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(54) Title: NANOPARTICULATE COMPOSITIONS COMPRISING INORGANIC CORES

(57) Abstract: The invention is directed to nanoparticulate compositions comprising inorganic cores and methods of making and using such compositions. The nonoparticulate compositions comprise at least one type of organic core having adsorbed or bound to the surface thereof at least one type of active molecule. The compositions exhibit superior properties as compared to conventional micronized and nanoparticulate active agent formulations.

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NANOPARTICULATE COMPOSITIONS COMPRISING INORGANIC CORES

The invention is directed to nanoparticulate compositions comprising inorganic cores and methods of making and using such compositions.

BACKGROUND

A. Background Related to Organic Nanoparticulate Compositions

Pharmaceutical agents that exhibit poor solubility often can diminish the efficacy of a drug formulation. Improved solubility can be achieved by reducing a drug's particle size, which increases its surface area.

Milling of pharmaceutical or diagnostic agents to a submicron particle size is described, for example, in U.S. Patent Nos. 5,145,684 "for Surface Modified Drug Nanoparticles;" 5,298,262 for "Use of Ionic Cloud Point Modifiers to Prevent Particle Aggregation During Sterilization;" 5,302,401 for "Method to Reduce Particle Size Growth During Lyophilization;" 5,318,767 for "X-Ray Contrast Compositions Useful in Medical Imaging;" 5,326,552 for "Novel Formulation For Nanoparticulate X-Ray Blood Pool Contrast Agents Using High Molecular Weight Non-ionic Surfactants;" 5,328,404 for "Method of X-Ray Imaging Using Iodinated Aromatic Propanedioates;" 5,336,507 for "Use of Charged Phospholipids to Reduce Nanoparticle Aggregation;" 5,340,564 for "Formulations Comprising Olin 10-G to Prevent Particle Aggregation and Increase Stability;" 5,346,702 for "Use of Non-Ionic Cloud Point Modifiers to Minimize Nanoparticulate Aggregation During Sterilization;" 5,349,957 for "Preparation and Magnetic Properties of Very Small Magnetic-Dextran Particles;" 5,352,459 for "Use of Purified Surface Modifiers to Prevent Particle Aggregation During Sterilization;" 5,399,363 for "Surface Modified Anticancer Nanoparticles;" 5,401,492 for "Water Insoluble Non-Magnetic Manganese Particles as Magnetic Resonance Enhancement Agents;" 5,429,824 for "Use of Tyloxapol as a Nanoparticulate Stabilizer;" 5,447,710 for "Method for Making Nanoparticulate X-Ray Blood Pool Contrast Agents Using

High Molecular Weight Non-ionic Surfactants;" 5,451,393 for "X-Ray Contrast Compositions Useful in Medical Imaging;" 5,466,440 for "Formulations of Oral Gastrointestinal Diagnostic X-Ray Contrast Agents in Combination with Pharmaceutically Acceptable Clays;" 5,470,583 for "Method of Preparing Nanoparticle Compositions Containing Charged Phospholipids to Reduce Aggregation;" 5,472,683 for "Nanoparticulate Diagnostic Mixed Carbamic Anhydrides as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" 5,494,683 for "Surface Modified Anticancer Nanoparticles;" 5,500,204 for "Nanoparticulate Diagnostic Dimers as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" 5,518,187 for "Method of Grinding Pharmaceutical Substances;" 5,518,738 for "Nanoparticulate NSAID Formulations;" 5,521,218 for "Nanoparticulate Iododipamide Derivatives for Use as X-Ray Contrast Agents;" 5,525,328 for "Nanoparticulate Diagnostic Diatrizoxy Ester X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" 5,543,133 for "Process of Preparing X-Ray Contrast Compositions Containing Nanoparticles;" 5,552,160 for "Surface Modified NSAID Nanoparticles;" 5,560,931 for "Formulations of Compounds as Nanoparticulate Dispersions in Digestible Oils or Fatty Acids;" 5,565,188 for "Polyalkylene Block Copolymers as Surface Modifiers for Nanoparticles;" 5,569,448 for "Sulfated Non-ionic Block Copolymer Surfactant as Stabilizer Coatings for Nanoparticle Compositions;" 5,571,536 for "Formulations of Compounds as Nanoparticulate Dispersions in Digestible Oils or Fatty Acids;" 5,573,749 for "Nanoparticulate Diagnostic Mixed Carboxylic Anydrides as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" 5,573,750 for "Diagnostic Imaging X-Ray Contrast Agents;" 5,573,783 for "Redispersible Nanoparticulate Film Matrices With Protective Overcoats;" 5,580,579 for "Site-specific Adhesion Within the GI Tract Using Nanoparticles Stabilized by High Molecular Weight, Linear Poly(ethylene Oxide) Polymers;" 5,585,108 for "Formulations of Oral Gastrointestinal Therapeutic Agents in Combination with Pharmaceutically Acceptable Clays;" 5,587,143 for "Butylene Oxide-Ethylene Oxide Block Copolymers Surfactants as Stabilizer Coatings for Nanoparticulate Compositions;" 5,591,456 for "Milled

Naproxen with Hydropropyl Cellulose as Dispersion Stabilizer;" 5,593,657 for "Novel Barium Salt Formulations Stabilized by Non-ionic and Anionic Stabilizers;" 5,622,938 for "Sugar Based Surfactant for Nanocrystals;" 5,628,981 for "Improved Formulations of Oral Gastrointestinal Diagnostic X-Ray Contrast Agents and Oral Gastrointestinal Therapeutic Agents;" 5,643,552 for "Nanoparticulate Diagnostic Mixed Carbonic Anhydrides as X-Ray Contrast Agents for Blood Pool and Lymphatic System Imaging;" 5,718,388 for "Continuous Method of Grinding Pharmaceutical Substances;" 5,718,919 for "Nanoparticles Containing the R(-)Enantiomer of Ibuprofen;" 5,747,001 for "Aerosols Containing Beclomethasone Nanoparticle Dispersions;" 5,834,025 for "Reduction of Intravenously Administered Nanoparticulate Formulation Induced Adverse Physiological Reactions;" 6,045,829 "Nanocrystalline Formulations of Human Immunodeficiency Virus (HIV) Protease Inhibitors Using Cellulosic Surface Stabilizers;" 6,068,858 for "Methods of Making Nanocrystalline Formulations of Human Immunodeficiency Virus (HIV) Protease Inhibitors Using Cellulosic Surface Stabilizers;" 6,153,225 for "Injectable Formulations of Nanoparticulate Naproxen;" 6,165,506 for "New Solid Dose Form of Nanoparticulate Naproxen;" 6,221,400 for "Methods of Treating Mammals Using Nanocrystalline Formulations of Human Immunodeficiency Virus (HIV) Protease Inhibitors," 6,264,922 for "Nebulized Aerosols Containing Nanoparticle Dispersions;" 6,267,989 for "Methods for Preventing Crystal Growth and Particle Aggregation in Nanoparticle Compositions;" 6,270,806 for "Use of PEG-Derivatized Lipids as Surface Stabilizers for Nanoparticulate Compositions;" 6,316,029 for "Rapidly Disintegrating Solid Oral Dosage Form," 6,375,986 for "Solid Dose Nanoparticulate Compositions Comprising a Synergistic Combination of a Polymeric Surface Stabilizer and Dioctyl Sodium Sulfosuccinate;" 6,428,814 for "Bioadhesive Nanoparticulate Compositions Having Cationic Surface Stabilizers;" and 6,432,381 for "Methods for Targeting Drug Delivery to the Upper and/or Lower Gastrointestinal Tract," all of which are specifically incorporated by reference. In addition, U.S. Patent Application No. 20020012675 A1, published on January 31, 2002,

for "Controlled Release Nanoparticulate Compositions," describes nanoparticulate compositions, and is specifically incorporated by reference.

Amorphous small particle compositions are described in, for example, U.S. Patent Nos. 4,783,484 for "Particulate Composition and Use Thereof as Antimicrobial Agent," 4,826,689 for "Method for Making Uniformly Sized Particles from Water-Insoluble Organic Compounds," 4,997,454 for "Method for Making Uniformly-Sized Particles From Insoluble Compounds," 5,741,522 for "Ultrasmall, Non-aggregated Porous Particles of Uniform Size for Entrapping Gas Bubbles Within and Methods," and 5,776,496, for "Ultrasmall Porous Particles for Enhancing Ultrasound Back Scatter.

B. Inorganic Nanoparticulate Compositions

The preparation of uniform drug particles with specific requirements in terms of size, shape, as well as chemical and physical properties, is of interest in the formulation of pharmaceutical products and in medical applications. The physical properties of these particles affect their dissolution, absorption rate, bioavailability, stability, uniformity, and other properties. Generally, ultrafine organic drug particles have been prepared by classical dry or wet milling processes in the presence of stabilizers. *See* the citations above; Liversidge et al., *Int. J. Pharm., 125*:91 (1995); and Liversidge et al., *Int. J. Pharm., 125*:309 (1995). However, these procedures usually yield dispersions of somewhat broad size distribution and cannot produce particles of desirable shapes.

Recently, it was demonstrated that uniform colloidal particles of naproxen and budesonide can be obtained in different morphologies by precipitation techniques. Goia et al., *J. Colloid Interface Sci., 206*:583 (1998); Pozarnsky et al., *Colloids Surf., 125*:47 (1997); Ruch et al., *J. Colloid Interface Sci., 229*:207 (2000). However, none of the employed methods produced monodispersed nanosized materials to yield a large enough specific surface area to be useful in this specific application. The present invention satisfies the need of producing a suspension having a large specific surface area by deposition of a pharmaceutically active ingredient onto a small inert core particle.

SUMMARY

The present invention is directed to the procedure by which nanoparticulate inorganic cores can be coated with pharmaceutically active agents to produce stable dispersions.

Because these nanoparticulate compositions have a smaller and more narrow particle size distribution than prior art nanoparticulate compositions, the compositions can have, for example, a more rapid dissolution rate, which may allow for better dose uniformity, higher bioavailability, and a faster onset of action. This is an improvement over prior art nanoparticulate technologies.

Moreover, in contrast to many prior art nanoparticulate compositions, a surface stabilizer may not be required to form stable and non-aggregated compositions. This is significant as compositions requiring fewer components can be useful in avoiding adverse reactions in *in vivo* use.

Another aspect of the invention is directed to pharmaceutical compositions comprising a nanoparticulate composition of the invention. The pharmaceutical composition comprises at least one nanoparticulate inorganic core, at least one active agent adsorbed or bound to the surface of the core, and a pharmaceutically acceptable carrier, as well as any desired excipients.

This invention further discloses methods of making a nanoparticulate composition having at least one inorganic core and adsorbed or bound to the surface thereof at least one active agent. Such a method comprises contacting at least one solubilized active agent with at least one suitable inorganic core for a time and under conditions until adsorption between the core and active agent occurs. Alternatively, at least one solubilized active agent is contacted with at least one suitable inorganic core in the presence of a coupling agent for a time and under conditions until binding between the core-coupling agent-active agent occurs.

The present invention is further directed to methods of treatment comprising administering to a mammal in need a therapeutically effective amount of a nanoparticulate composition according to the invention.

Yet another aspect of the invention is directed to the application of the described nanoparticulate compositions to any biological surface of an animal. Such application encompasses, for example, the application of nanoparticulate compositions useful as cosmetics, perfumes, shampoos, cleansers, moisturizers, deodorants, topical creams, ointments, nail polish, hair cosmetic compositions, *etc.*

The compositions of the invention can also be applied to plant tissue. Such methods include applying nanoparticulate compositions useful as fertilizers, pesticides, herbicides, *etc.* to a biological surface of a plant.

Both the foregoing general description and the following detailed description are exemplary and explanatory and are intended to provide further explanation of the invention as claimed. Other objects, advantages, and novel features will be readily apparent to those skilled in the art from the following detailed description of the invention.

DESCRIPTION OF THE FIGURES

Figure 1: Shows ζ-potential as a function of the pH of: (a) Nalco alumina (O); and (b) DGC alumina (Δ) at C_{NaCl} mol dm^{-3};

Figure 2: Shows ζ-potential as a function of the concentrations of naproxen and ketoprofen (mol dm^{-3}) of: (a) DGC/naproxen in water (O); (b) Nalco/naproxen in water (Δ); (c) DGC/ketoprofen in water (•) at pH = 7.2 ±0.1 in $C_{NaCl} = 10^{-3}$ mol dm^{-3}; (d) DGC/naproxen in ethanol (V); and (e) DGC/ketoprofen in ethanol (▼);

Figure 3: Shows adsorbed amount of naproxen and ketoprofen T (μmol m^{-2}) as a function of their concentration (mol dm^{-3}) for: (a) DGC/naproxen in water (O); (b) DGC/ketoprofen in water (•) at pH = 7.2 ±0.1; (c)

DGC/naproxen in ethanol (V); and DGC/ketoprofen in ethanol (▼);

Figure 4: Shows ATR-FTIR spectra of: (a) DGC alumina; (b) naproxen; and (c) DGC alumina coated with naproxen in water;

Figure 5: Shows Transmission Electron Microscopy (TEM) pictures of: (a) Nalco alumina; and (b) Nalco alumina coated with naproxen;

Figure 6: Shows FTIR spectra of: (a) naproxen; (b) Ludox CL® (2 wt%) + naproxen (0.01 mol. dm^{-3} at pH = 8.55 in water; and (c) Ludox CL® silica;

Figure 7: Shows FTIR spectra of: (a) naproxen; (b) Ludox CL® (2 wt%) + naproxen (0.01 mol. dm^{-3} in ethanol; and (c) Ludox CL® silica;

Figure 8: Shows FTIR spectra of: (a) naproxen; (b) Nissan silica (2 wt%) + naproxen (0.01 mol. dm^{-3} in methanol; and (c) Nissan silica;

Figure 9: Shows FTIR spectra of: (a) naproxen; (b) Nalco alumina (0.1 wt%) + naproxen (0.0025 mol. dm^{-3} in water; and (c) alumina Nalco;

Figure 10: Shows shows FTIR spectra of: (a) naproxen; (b) Nalco alumina (2 wt%) + naproxen (0.05 mol. dm^{-3} in methanol; and (c) alumina Nalco;

Figure 11: Shows the ζ-potentials of both alumina cores, Nalco alumina (O) and Degussa C alumina (□) in 1×10^{-3} mol dm^{-3} aqueous NaCl solution, as a function of the pH;

Figure 12: Shows the ζ -potentials of both alumina cores, Nalco alumina (O) and Degussa C alumina (□), in 1×10^{-3} mol dm^{-3} aqueous NaCl solution at a pH of 7.2 ± 0.1, and of Degussa C alumina in ethanol (Δ) as a function of the concentration of added naproxen;

Figure 13: Shows the adsorption isotherms of naproxen on Degussa C alumina in water (□) at a pH of 7.2 ± 0.1 and in ethanol (Δ);

Figure 14: Shows the FTIR spectra of Degussa C alumina (a), naproxen (b), and Degussa C alumina coated with naproxen in ethanol (c);

Figure 15: Shows the FTIR spectra of Nalco alumina (a); naproxen (b); and Nalco alumina coated with naproxen in water (c);

Figure 16: Shows transmission electron micrographs of the Nalco alumina core (a) and of Nalco alumina core coated with naproxen (b);

Figure 17: Shows transmission electron micrographs of the Degussa C alumina core (a) and of the same particles coated with naproxen (b);

Figure 18: Shows the ζ-potentials of Degussa C alumina (□) in 1 $x10^{-3}$ mol dm^{-3} NaCl aqueous solution at pH = 7.2 ± 0.1, and of Degussa C alumina in ethanol (Δ) as a function of the concentration of added ketoprofen;

Figure 19: Shows the adsorption data of ketoprofen on Degussa C alumina in water (pH 7.2 ± 0.1) (□) and in ethanol (Δ) (the concentration of alumina in all samples was 0.05 wt%);

Figure 20: Shows the FTIR spectra of Degussa C alumina (a), ketoprofen (b), and Degussa C alumina coated with ketoprofen in ethanol (c);

Figure 21: Shows a TEM of Degussa C alumina coated with ketoprofen;

Figure 22: Shows a SEM of silica MP4540;

Figure 23: Shows a SEM of silica (MP4540) equilabrated with cyclosporin (1 x 10^{-3} mol dm^{-3});

Figure 24: Shows FTIR spectra of silica (MP4540) (a), cyclosporin (b), and silica equilibrated with cyclosporin (1 $x10^{-3}$ mol dm^{-3}) (c);

Figure 25: Shows a SEM of hematite particles;

Figure 26: Shows a SEM of hematite particles after equilabration with cyclosporin (5 x 10^{-3} mol dm^{-3}); and

Figure 27: Shows FTIR spectra of Ludox AM silica (a); ketoprofen (b) modified silica with N-phenylaminopropyltrimethoxysilane (c), and modified silica coated with ketoprofen in water pH = 7.1 (d).

DETAILED DESCRIPTION OF THE INVENTION

I. Nanoparticulate Compositions

The present invention is directed to novel compositions comprising at least one type of inorganic core having adsorbed or bound to the surface thereof at least one type of active molecule. The compositions exhibit superior properties as compared to conventional micronized and nanoparticulate active agent formulations.

The particle size of the compositions can be narrowly defined based on the particle size of the nanoparticulate inorganic core. This enables the preparation of nanoparticulate compositions having a very narrow particle size range, which is preferred as the particle size of a composition affects the dissolution rate, and thus bioavailability, of a composition.

In addition, more precise controlled release formulations can be made using the technology of the invention. Specifically, compositions comprising different size inorganic cores can be made in which the smaller inorganic cores release drug at a faster rate than the larger inorganic cores. Such formulations are useful in the pharmaceutical field, as well as, for example, in pesticides, insecticides, fertilizers, and personal care products.

The nanoparticulate compositions are useful in a wide variety of applications. For example, the nanoparticulate compositions of the invention can be used in pharmaceuticals, including biologics such as proteins and peptides, organic compounds, such as therapeutic small molecules, agricultural agents, cosmetic agents, hair compositions, and others.

A. Inorganic Cores

The inorganic cores of the invention comprise a suitable inorganic material having a nanoparticulate particle size. The inorganic material can vary depending upon the intended use of the resulting composition. For example, compositions intended to be used as pharmaceutical formulations will have different requirements than those

intended for agricultural, veterinary, or other uses. Exemplary inorganic cores, suitable for pharmaceutical and other uses, are nanoparticulate silica, alumina, and hematite.

The general class of inorganic compounds may be expressed as metal oxides, metal hydroxides, metal hydrous oxides, metal sulfides, metal sulfates, metal carbonates, metal phosphates, *etc.* For the core to be useful in a pharmaceutical composition, it must be non-toxic, reactive to the coupling agent or the drug, and be available as colloid particles in the specified particle size range. For all applications, the core must be reactive to the coupling agent or active agent, and be available as colloid particles in the specified particle size range.

Other exemplary inorganic core materials include, but are not limited to, titanium dioxide, zinc oxide, chromium oxide, chromium hydroxide, and silver halides. Furthermore, cadmium oxide, cadmium sulfide, zinc sulfide, copper oxide, barium sulfate, cerium oxide, and cobalt oxide may also be used (several of the latter inorganic cores may exhibit toxicity which prevent their use in a pharmaceutical application).

Preferably the inorganic cores have a particle size of less than about 1 micron, less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 200 nm, less than about 100 nm, less than about 75 nm, less than about 50 nm, less than about 25 nm, less than about 15 nm, less than about 10 nm, or less than about 5 nm.

Particle size, as used in this invention, refers to an "average" particle size in which at least 50% of the particles are less than the recited size. Other embodiments of the invention encompass a particle size in which at least 60%, 70%, 80%, or 90% of the particles are less than the recited size. For example, for an inorganic core particle size of 10 nm, at least 50% of the inorganic cores of the composition are less than 10 nm. In other embodiments of the invention, at least 60%, 70%, 80%, or 90% of the inorganic cores are less than 10 nm.

B. Coupling Agents

If a solubilized active agent does not adsorb to the surface of an inorganic core, a coupling agent can be used to form the nanoparticulate compositions of the invention. Suitable coupling agents are, for example, N-phenylaminopropyltrimethoxysilane, 3-aminopropylmethyldiethoxysilane, and methacryloxypropyltrimethoxysilane. The general class of useful coupling agents may be expressed as, but not limited to, amino silanes and caroxy silanes.

C. Active Agents

Any active agent may be used in the invention. The active agent must be substantially soluble in at least one liquid medium. The invention can be practiced with liquid media in which a drug substance is substantially soluble including, for example, water, aqueous salt solutions, safflower oil, and solvents such as ethanol, t-butanol, hexane and glycol. The pH of the dispersion media can be adjusted by techniques known in the art.

The active agent can be a drug, which is preferably present in an essentially pure form. A drug can be selected from a variety of known classes of drugs, as provided in U.S. Patent No. 5,145,684, including, for example, proteins, peptides, nutraceuticals, anti-obesity agents, corticosteroids, elastase inhibitors, analgesics, anti-fungals, oncology therapies, anti-emetics, analgesics, cardiovascular agents, anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, antibiotics (including penicillins), anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytic sedatives (hypnotics and neuroleptics), astringents, beta-adrenoceptor blocking agents, blood products and substitutes, cardiac inotropic agents, contrast media, cough suppressants (expectorants and mucolytics), diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics (antiparkinsonian agents), haemostatics, immunological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin and

biphosphonates, prostaglandins, radio- pharmaceuticals, sex hormones (including steroids), anti-allergic agents, stimulants and anoretics, sympathomimetics, thyroid agents, vasodilators and xanthines.

Exemplary nutraceuticals and dietary supplements are disclosed, for example, in Roberts et al., *Nutraceuticals: The Complete Encyclopedia of Supplements, Herbs, Vitamins, and Healing Foods* (American Nutraceutical Association, 2001), which is specifically incorporated by reference. A nutraceutical or dietary supplement, also known as phytochemicals or functional foods, is generally any one of a class of dietary supplements, vitamins, minerals, herbs, or healing foods that have medical or pharmaceutical effects on the body. Exemplary nutraceuticals or dietary supplements include, but are not limited to, folic acid, fatty acids (*e.g.*, DHA and ARA), fruit and vegetable extracts, vitamin and mineral supplements, phosphatidylserine, lipoic acid, melatonin, glucosamine/chondroitin, Aloe Vera, Guggul, glutamine, amino acids (*e.g.*, iso-leucine, leucine, lysine, methionine, phenylanine, threonine, tryptophan, and valine), green tea, lycopene, whole foods, food additives, herbs, phytonutrients, antioxidants, flavonoid constituents of fruits, evening primrose oil, flax seeds, fish and marine animal oils, and probiotics. Nutraceuticals and dietary supplements also include bio-engineered foods genetically engineered to have a desired property, also known as "pharmafoods."

Drugs to be administered in an aerosol formulation are preferably selected from the group consisting of proteins, peptides, bronchodilators, corticosteroids, elastase inhibitors, analgesics, anti-fungals, cystic-fibrosis therapies, asthma therapies, emphysema therapies, respiratory distress syndrome therapies, chronic bronchitis therapies, chronic obstructive pulmonary disease therapies, organ-transplant rejection therapies, therapies for tuberculosis and other infections of the lung, fungal infection therapies, respiratory illness therapies associated with acquired immune deficiency syndrome, oncology drugs, anti-emetics, analgesics, and cardiovascular agents.

The active ingredient may be present in any amount which is sufficient to elicit a therapeutic effect and, where applicable, may be present either substantially in the form of one optically pure enantiomer or as a mixture, racemic or otherwise, of enantiomers.

The active agents are commercially available and/or can be prepared by techniques known in the art.

1. Active Agents Useful in Dermal Applications

The active agents according to the present invention include but are not limited to active agents which can be used in dermal applications, *e.g.*, sunscreens, cosmetics, topical application of pharmaceuticals to the dermis (acne medication, anti-wrinkle drugs, such as alpha-hydroxy formulations), nail polish, moisturizers, deodorant, *etc.*

Cosmetic compositions are generally defined as compositions suitable for application to the human body. Cosmetic compositions such as creams and lotions are used to moisturize the skin and keep it in a smooth, supple condition. Pigmented cosmetic compositions, such as makeup, blush, lipstick, and eye shadow, are used to color the skin and lips. Since color is one of the most important reasons for wearing cosmetics, color-containing cosmetics must be carefully formulated to provide maximum wear and effect.

Other areas which benefit from the present invention include coloring agents, flavors and fragrances. Coloring agents or pigments are used in cosmetic applications as well as in fabric applications. Suitable pigments can be inorganic and/or organic. Also included within the term pigment are materials having a low color or luster, such as matte finishing agents, and also light scattering agents. Examples of suitable pigments are iron oxides, acylglutamate iron oxides, ultramarine blue, D&C dyes, carmine, and mixtures thereof. Depending upon the type of cosmetic composition, *e.g.*, foundation or blusher, a mixture of pigments will normally be used.

Fragrances and odiferous compounds are also suitable for use in the present inventive compositions. Fragrances or perfumes are usually prepared from volatile oils distilled or extracted from the leaves, flowers, gums, or woods of plant life (occasionally from animal life). These include, for example, linalyl acetate from citral, jasmine, cedar, lavender, and attar of rose. A typical fragrance may consist of many volatile components blended to create a pleasant sensory experience to the person wearing the

fragrance and also impart a pleasant sensory experience to the people around that person. These blended oils, however, are typically too potent or too expensive to wear without being diluted in an appropriate solvent. Present perfumeries use lower molecular weight alcohol, *e.g.*, methanol or ethanol, and more typically ethanol, to prepare a variety of "perfume" products, such as eau de cologne, perfume, eau de parfum, eau de toilette, splash cologne, and eau fraiche for the consumer. Nanoparticulate compositions comprising a fragrance or odiferous compound as an active agent could provide prolonged sensory stimulation following application; *i.e.*, for up to 48 hours following application to the skin.

2. Active Agents Useful in Mucous Applications

Exemplary active agents to be applied to mucous include dental applications, such as oral nanoparticulate lidocain formulations, nanoparticulate fluoride treatments, application to the lungs, throat, gastrointestinal tract (GIT), application to wounds, *etc.* Also included is application to the throat using a liquid containing a nanoparticulate formulation containing, for example, menthol or other numbing compound for treatment of coughs or sore throats. The stomach and GIT can also be treated using nanoparticulate formulations. This is particularly useful for treatment of diseases associated with the mucous of the gastrointestinal tract, such as Crohn's Disease.

Other pharmaceutical therapeutic methodologies include colonic, oral, rectal, intravaginal, injectable (e.g., intravenous or subcutaneous), pulmonary, nasal, buccal, topical, local, intracisternal, intraperitoneal, ocular, aural, transdermal, buccal spray, or nasal spray administration.

The compositions of the invention also encompass food products. For example, spice, oleoresin, flavor oil, color, or chemicals are often added during food processing to produce the desirable flavors, taste, and appearance. These agents can be included in a nanoparticulate composition of the present invention for increased adhesion to biological surfaces. Nanoparticulate flavoring agents could be used in products such as

gums to produce prolonged flavor.

3. Active Agents Useful in Hair Applications

Biological substrates such as the hair are also encompassed by the scope of the invention. Nanoparticulate compositions can be used in hair conditioner formulations, hair dyes, hair sprays, hair cosmetics, hair cleansers, depilatories, *etc.*

4. Active Agents Useful in Plant Tissue Applications

Yet another area of applicability of the present invention includes nanoparticulate compositions that can be applied to plant tissue.

Nanoparticulate compositions can be used for applications of pesticides, insecticides, fertilizers, *etc.* – any substance to be applied to the surface of a plant. All plants, such as grass, trees, commercial farm crops (such as corn, soybeans, cotton, vegetables, fruit, *etc*), weeds, *etc.*, are encompassed by the scope of this invention.

In one embodiment of the invention, the active agent of the nanoparticulate composition is an insecticidal ingredient applied to seeds, plants, trees, harvested crops, soil, and the like. The insecticide ingredient can be selected from a wide variety of organic compounds or mixtures which are known and used in agriculture and horticulture applications, such as those listed in W. T. Thomson, *Agricultural Chemicals, Book I, Insecticides* (Thomson Publications, Fresno, Calif. 1989).

The general categories of insecticidal-active organic compounds include chlorinated hydrocarbon derivatives, phosphorated derivatives, pyrethroids, acylureas, and the like. Chlorinated hydrocarbon insecticides usually act as stomach and contact poisons affecting the nervous system. They are persistent in the environment and tend to accumulate in animal fatty tissue, as exemplified by DDT and chlordane.

Illustrative of other insecticidal compounds are chlorfluazuron, chlorpyrifos, chlorpyrifos methyl, bromophos, diazinon, malathion, trichlorfon, dimethoate, phorate, lindane, toxaphene, diflubenuron, methomyl, propoxur, carbaryl, cyhexatin, cypermethrin, permethrin, fenvalerate, dicofol, tetradifon, propargite, and the like.

Other examples of insecticides include the pyrethroid insecticides, such a Fenvalerate™ [α-cyano-3-phenoxybenzyl-2-(4-chlorophenyl)-3methylvalerate] and Pyrethroid™ [cyano(4-fluoro-3-phenoxyphenylmethyl-3-(2,2-dichloroethenyl)-2,2-dimethyl cyclopropanecarboxylate]; organophosphorus insecticides, such as DDVP™ (2,2-dichlorovinyldimethyl phosphate), Sumithion™ (dimethyl-4-nitro-m-tolylphosphorothionate), Malathone™ {S-[1,2-bis(ethoxycarbonyl)ethyl]dimethyl-phosphorothiol thionate}, Dimethoate [dimethyl-S-(N-methylcarbamoylmethyl)-phosphorothios thionate), Elsan™ {S-[.alpha.-(ethoxycarbonyl)benzyl] dimethylphosphorothiol thionate), and Baycid™ [O,O-dimethyl-O-(3-methyl-4methylmercaptophenyl)thiophosphate]; carbamate; insecticides such as Bassa™ (O-butylphenyl methylcarbamate), MTMC™ (m-tolyl methylcarbamate), Meobal™ (3,4-dimethylphenyl-N-methylcarbamate), and NAC™ (1-naphthyl-N-methylcarbamate); as well as Methomyl™ {methyl-N[(methylcarbamoyl)oxy]thioacetimide} and Cartap™ {1,3-bis(carbamolythio)-2-(N,N-dimethylamino)propane hydrochloride}.

Examples of other agricultural agents include acaricides such as, but not limited to, Smite™ {2-[2-(p-tert-butylphenoxy)isopropoxy]isopropyl-2-chloroethyl sulfide}, Acricid™ (2,4-dinitro-6-sec-butylphenyl dimethylacrylate), Chlormit™ (isopropyl 4,4-dichlorobenzylate), Acar™ (ethyl 4,4-dichlorobenzylate), Kelthane™ [1,1-bis(p-chlorophenyl)-2,2,2-trichloroethanol], Citrazon™ (ethyl O-benzoyl-3-chloro-2,6-dimethoxybenzohydroxymate), Plictran™ (tricyclohexyltin hydroxide), and Omite™ [2-(p-tert-butylphenoxy)cyclohexyl-2-propinyl sulfite].

Examples of germicides include organosulfur germicides, such as Dithane™ (zinc ethylenebisdithiocarbamate), Maneo™ (manganese ethylenebis-dithiocarbamate), Thiuram™ [bis(dimethylthiocarbamoyl) disulfide], Benlate™ [methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate], Difolatan™ (N-tetrachloroethylthio-4-cyclohexane-1,2-dicarboxyimide), Daconol™ (tetrachloroisophthalonitrile), Pansoil™ (5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole), Thiophanate-methyl[1,2-bis(3-methoxycarbonyl-2-thioureido)benzene], Rabcide™ (4,5,6,7-tetrachlorophthaloid),

Kitazin P™ (O,O-diisopropyl-S-benzyl phosphorothioate), Hinonsan™ (O-ethyl-S,S-diphenyldithiophosphate), and Propenazol™ (3-allyloxy-1,2-benzothiazole 1,1-dioxide).

Example of plant growth regulating agents include, but are not limited to, MH™ (maleic acid hydrazide) and Ethrel™ (2-chloroethylphosphonic acid).

Examples of herbicides include, but are not limited to Stam™ (3,4-dichloropropionanilide), Saturn™ [S-(4-chlorobenzyl) N,N-diethylthiolcarbamate), Lasso (2-chloro-2',6'-diethyl-N-(methoxymethyl)acetanilide), Glyphosate™ [N-(phosphonomethyl)glycine isopropylamine salt], DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea), and Gramoxone™ (1,1'-dimethyl-4,4'-dipyridium dichloride].

Other herbicides contemplated for use in the present invention include auxin transport inhibitors, *e.g.*, naptalam; growth regulators, including benzoic acids, *e.g.*, dicamba; phenoxy acids, such as (i) acetic acid type, *e.g.*, 2,4-D, MCPA, (ii) propionic acid type, *e.g.*, 2,4-DP, MCPP, and (iii) butyric acid type, *e.g.*, 2,4-DB, MCPB; picolinic acids and related compounds, *e.g.*, picloram, triclopyr, fluroxypyr, and clopyralid.

Photosynthesis inhibitors are also herbicides useful in the compositions of the invention. Such compounds include but are not limited to (a) s-triazines, such as (i) chloro substituted, *e.g.*, atrazine, simazine, and cyanazine, (ii) methoxy substituted, *e.g.*, prometon, (iii) methylthio substituted, *e.g.*, ametryn and prometryn; (b) other triazines, such as hexazinone, and metribuzin; (c) substituted ureas, such as diuron, fluometuron, linuron, tebuthiuron, thidiazuron, and forchlorfenuron; (d) uracils, such as bromacil and terbacil; and (e) others, such as bentazon, desmedipham, pheninedipham, propanil, pyrazon, and pyridate.

Pigment inhibitors are also herbicides useful in the compositions of the invention. Such compounds include but are not limited to pyridazinones, such as norflurazon; isoxazolones, such as clomazone; and others, such as amitrole and fluridone.

In yet another aspect of the invention, growth inhibitors are herbicides useful in the compositions of the invention. Such compounds include but are not limited to (a) mitotic disruptors, such as (i) dinitroanilines, *e.g.*, trifluralin, prodiamine, benefin,

ethalfluralin, isopropalin, oryzalin, and pendimethalin; and (ii) others, such as DCPA, dithiopyr, thiazopyr, and pronamide; (b) inhibitors of shoots of emerging seedlings, such as (i) thiocarbamates, *e.g.*, EPTC, butylate, cycloate, molinate, pebulate, thiobencarb, triallate, and vernolate; (c) inhibitors of roots only of seedlings, such as bensulide, napropamide, and siduron; and (d) inhibitors of roots and shoots of seedlings, including chloroacetamides, such as alachlor, acetochlor, metolachlor, diethatyl, propachlor, butachlor, pretilachlor, metazachlor, dimethachlor, and cinmethylin.

Amino acid synthesis inhibitors are herbicides useful in the compositions of the invention. Such compounds include, but are not limited to, (a) glyphosate, glufosinate; (b) sulfonylureas, such as rimsulfuron, metsulfuron, nicosulfuron, triasulfuron, primisulfuron, bensulfuron, chlorimuron, chlorsulfuron, sulfometuron, thifensulfuron, tribenuron, ethametsulfuron, triflusulfuron, clopyrasulfuron, pyrazasulfuron, prosulfuron (CGA-152005), halosulfuron, metsulfuron-methyl, and chlorimuron-ethyl; (c) sulfonamides, such as flumetsulam (a.k.a. DE498); (d) imidazolinones, such as imazaquin, imazamethabenz, imazapyr, imazethapyr, and imazmethapyr.

Lipid biosynthesis inhibitors are herbicides useful in the compositions of the invention. Such compounds include, but are not limited to, (a) cyclohexanediones, such as sethoxydim and clethodim; (b) aryloxyphenoxys, such as fluazifop-(P-butyl), diclofop-methyl, haloxyfop-methyl, and quizalofop; and (c) others, such as fenoxaprop-ethyl.

Cell wall biosynthesis inhibitors are herbicides useful in the compositions of the invention. Such compounds include, but are not limited to, dichlobenil and isoxaben.

Rapid cell membrane disruptors are herbicides useful in the compositions of the invention. Such compounds include, but are not limited to, (a) bipyridiliums, such as paraquat, and diquat; (b) diphenyl ethers, such as acifluorfen, fomesafen, lactofen, and oxyfluorfen; (c) glutamine synthetase inhibitors, such as glufosinate; and (d) others, such as oxadiazon.

Miscellaneous herbicides useful in the compositions of the invention include, but are not limited to, (a) carbamates, such as asulam; (b) nitriles, such as bromoxynil and

ioxynil; (c) hydantocidin and derivatives; and (d) various other compounds, such as paclobutrazol, ethofumesate, quinclorac (a.k.a. BAS514), difenzoquat. endothall, fosamine, DSMA, and MSMA.

Other herbicides useful in the compositions of the invention include, but are not limited to, triketones and diones of the type described in U.S. Patent Nos. 5,336,662 and 5,608,101, the contents of each of which are incorporated herein by reference, and in EP-A-338-992; EP-A-394-889; EP-A-506,967; EP-A-137,963; EP-A-186-118; EP-A-186-119; EP-A-186-120; EP-A-249-150; and EP-A-336-898. Examples of such triketones and diones are sulcotrione (MIKADO™), whose chemical designation is 2-(2-chloro-4-methanesulfonylbenzoyl)-1,3-cyclohexanedione: 2-(4-methylsulfonyloxy-2-nitrobenzoyl)-4,4,6,6-tetramethyl-1,3-cyclohexane dione; 3-(4-methylsulfonyloxy-2-nitrobenzoyl)-bicyclo[3,2,1]octane-2,4-dione3-(4- methylsulfonyl-2-nitrobenzoyl)-bicyclo[3,2,1]octane-2,4-dione; 4-(4-chloro-2-nitrobenzoyl)-2,6,6-trimethyl-2H-1,2-oxazine-3,5(4H,6H)dione ; 4-(4-methylthio-2-nitrobenzoyl)-2,6,6-trimethyl-2H-1,2-oxazine-3,5(4H,6H) -dione; 3-(4-methylthio-2-nitrobenzoyl)-bicyclo[3,2,1]octane-2,4-dione; 4-(2-nitro-4-trifluoromethoxybenzoyl)-2,6,6-trimethyl-2H-1,2-oxazine-3,5(4 H,6H)-dione.

Herbicidal compounds useful in the nanoparticulate compositions of the invention are described in U.S. Patent No. 5,506,192; EP-A-461,079; EP-A-549,524; EP-A-315,589 and PCT Appln. No. 91/10653. The contents of all of the cited references are incorporated herein by reference; including for example 3-[(4,6-dimethoxy-2-pyrimidinyl)hydroxymethyl]-N-methyl-2-pyridine carboxamide; 4,7-dichloro-3-(4,6-dimethoxy-2-pyrimidinyl)-3-hexanoyloxyphthalide; 3-[(4,6-dimethoxy-2-pyrimidinyl)carbonyl]-N,N-dimethyl-2-pyridine carboxamide; 3,6-dichloro-2-[(4,6-dimethoxy-2-pyrimidinyl)carbonyl]benzoic acid; 6-chloro-2-[(4,6-dimethoxy-2-pyrimidinyl)thio]benzoic acid (a.k.a. DPX-PE350 or pyrithiobac) and salts thereof.

5. Agents Useful in Miscellaneous Applications

Other exemplary uses of the novel nanoparticulate formulations are provided: teeth can be treated with teeth whiteners or fluoride nanoparticulate compositions; bones can be treated with calcium nanoparticulate compositions; nails can be treated with color or strengthening nanoparticulate formulations; insects or pests can be treated with insecticides or other toxic compositions to the pest.

D. Other Pharmaceutical Excipients

Pharmaceutical compositions according to the invention may also comprise one or more binding agents, filling agents, lubricating agents, suspending agents, sweeteners, flavoring agents, preservatives, buffers, wetting agents, disintegrants, effervescent agents, and other excipients. Such excipients are known in the art.

Examples of filling agents are lactose monohydrate, lactose anhydrous, and various starches; examples of binding agents are various celluloses and cross-linked polyvinylpyrrolidone, microcrystalline cellulose, such as Avicel® PH101 and Avicel® PH102, microcrystalline cellulose, and silicified microcrystalline cellulose (SMCC).

Suitable lubricants, including agents that act on the flowability of the powder to be compressed, are colloidal silicon dioxide, such as Aerosil® 200, talc, stearic acid, magnesium stearate, calcium stearate, and silica gel.

Examples of sweeteners are any natural or artificial sweetener, such as sucrose, xylitol, sodium saccharin, cyclamate, aspartame, and acsulfame. Examples of flavoring agents are Magnasweet® (trademark of MAFCO), bubble gum flavor, and fruit flavors, and the like.

Examples of preservatives are potassium sorbate, methylparaben, propylparaben, benzoic acid and its salts, other esters of parahydroxybenzoic acid such as butylparaben, alcohols such as ethyl or benzyl alcohol, phenolic compounds such as phenol, or quarternary compounds such as benzalkonium chloride.

Suitable diluents include pharmaceutically acceptable inert fillers, such as microcrystalline cellulose, lactose, dibasic calcium phosphate, saccharides, and/or mixtures of any of the foregoing. Examples of diluents include microcrystalline

cellulose, such as Avicel® PH101 and Avicel® PH102; lactose such as lactose monohydrate, lactose anhydrous, and Pharmatose® DCL21; dibasic calcium phosphate such as Emcompress®; mannitol; starch; sorbitol; sucrose; and glucose.

Suitable disintegrants include lightly crosslinked polyvinyl pyrrolidone, corn starch, potato starch, maize starch, and modified starches, croscarmellose sodium, cross-povidone, sodium starch glycolate, and mixtures thereof.

Examples of effervescent agents are effervescent couples such as an organic acid and a carbonate or bicarbonate. Suitable organic acids include, for example, citric, tartaric, malic, fumaric, adipic, succinic, and alginic acids and anhydrides and acid salts. Suitable carbonates and bicarbonates include, for example, sodium carbonate, sodium bicarbonate, potassium carbonate, potassium bicarbonate, magnesium carbonate, sodium glycine carbonate, L-lysine carbonate, and arginine carbonate. Alternatively, only the sodium bicarbonate component of the effervescent couple may be present.

E. Concentration of Nanoparticulate Inorganic Core and Active Agent

The relative amount of at least one inorganic core and one or more active agents can vary widely. The optimal amount of the active agent can depend, for example, upon the particular active agent selected and the intended use of the nanoparticulate composition.

The concentration of the one or more inorganic cores can vary from about 0.1% to about 99.9% by weight based on the total combined dry weight of the inorganic core and active agent.

The concentration of the at least one active agent can vary from about 0.001% to about 99.9% by weight based on the total combined dry weight of the inorganic core and active agent.

II. Methods of Making Nanoparticulate Formulations

The nanoparticulate compositions of the invention can be made by contacting at least one solubilized active agent with at least one suitable inorganic core for a time and under conditions until adsorption between the core and active agent occurs. Alternatively, at least one solubilized active agent is contacted with at least one suitable inorganic core in the presence of a coupling agent for a time and under conditions until binding between the core-coupling agent-active agent occurs.

III. Methods of Using the Nanoparticulate Compositions of the Invention

The nanoparticulate compositions of the present invention can be administered to humans and animals in any pharmaceutically acceptable manner, such as orally, via pulmonary route, rectally, parenterally (intravenous, intramuscular, or subcutaneous), intracisternally, intravaginally, intraperitoneally, locally/topically (powders, ointments or drops), or as a buccal or nasal spray.

Such a method comprises administering to an animal or human in need a therapeutically effective amount of a nanoparticulate composition according to the invention.

The compositions can be applied to the surface of hair by spraying or soaking, as well as by other techniques known to those skilled in the art. The compositions can be applied to plant tissue by spraying, soaking, soil drench, pre-emergence and post-emergence, as well as by other techniques known to those skilled in the art.

Compositions suitable for parenteral injection may comprise physiologically acceptable sterile aqueous or nonaqueous dispersions, suspensions or emulsions and sterile powders for reconstitution into sterile injectable solutions or dispersions. Examples of suitable aqueous and nonaqueous carriers, diluents, solvents, or vehicles include water, ethanol, polyols (propyleneglycol, polyethyleneglycol, glycerol, and the like), suitable mixtures thereof, vegetable oils (such as olive oil), and injectable organic esters such as ethyl oleate.

Solid dosage forms for oral administration of a pharmaceutical active agent include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound is admixed with at least one of the following: (a) one or more inert excipients (or carrier), such as dicalcium phosphate; (b) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and silicic acid; (c) binders, such as carboxymethylcellulose, alignates, gelatin, polyvinylpyrrolidone, sucrose and acacia; (d) humectants, such as glycerol; (e) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain complex silicates, and sodium carbonate; (f) solution retarders, such as paraffin; (g) absorption accelerators, such as quaternary ammonium compounds; (h) wetting agents, such as cetyl alcohol and glycerol monostearate; (i) adsorbents, such as kaolin and bentonite; and (j) lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, or mixtures thereof. For capsules, tablets, and pills, the dosage forms may also comprise buffering agents.

Liquid application forms include emulsions, suspensions, syrups, and elixirs. In addition to the active compounds, the liquid dosage forms may comprise inert diluents commonly used in the art, such as water or other solvents, solubilizing agents, and emulsifiers. Exemplary emulsifiers are ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propyleneglycol, 1,3-butyleneglycol, dimethylformamide, oils, such as cottonseed oil, groundnut oil, corn germ oil, olive oil, castor oil, and sesame oil, glycerol, tetrahydrofurfuryl alcohol, polyethyleneglycols, fatty acid esters of sorbitan, or mixtures of these substances, and the like.

Besides such inert diluents, the composition can also include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

Actual application levels of active ingredients in the nanoparticulate compositions of the invention may be varied to obtain an amount of active ingredient that is effective to obtain a desired response for a particular composition and method of

application. The selected dosage level therefore depends upon the desired therapeutic effect, on the route of administration, on the desired duration of treatment, and other factors. In addition, the formulations of the present invention can be administered in combination with other pharmaceutical agents in the form of a solution, suspension, syrup or elixir or as formulated for solid dose administration.

The total daily amount of the active agent included in the inventive composition can be applied to a host in single or divided doses. Individuated units may contain such amounts of such submultiples thereof as may be used to make up the daily dose. It will be understood, however, that the specific dose level for any particular patient will depend upon a variety of factors, for example, when the host is a patient, such factors include the body weight, general health, sex, diet, time and route of administration, rates of absorption and excretion, combination with other drugs and the severity of the particular disease being treated.

The following examples are given to illustrate the present invention. It should be understood, however, that the invention is not to be limited to the specific conditions or details described in these examples. Throughout the specification, any and all references to a publicly available document, including a U.S. patent, are specifically incorporated by reference.

Example 1

The purpose of this example was to prepare nanoparticulate alumina particles coated with naproxen or ketoprofen.

Nanoparticulate alumina particles having modal diameters of 8 and 13 nm were successfully coated by adsorption of naproxen [(+)-6-methoxy-α-methyl-2-naphthalene-acetic acid] or ketoprofen [α-methyl-3-(4-methylbenzoil) benzene acetic acid] in aqueous and ethanol solutions. The presence of the drugs at the alumina surface was confirmed by attenuated total reflection infrared spectroscopy and electrokinetic measurements, while the bound amounts were assessed by thermogravimetric analysis.

Experimental: Preparation of Naproxen Particles

Materials

All organic chemicals were reagent grade and were not further purified. Naproxen (MW: 230 g mol^{-1}) was purchased from Aldrich Chemicals

Two commercially available alumina nanoparticles were used in the experiment. Degussa C (DGC) alumina, γ-Al_2O_3, (particle size about 13 nm, specific surface area (SSA) of 100 ± 10 m^2g^{-1}) was obtained in powder form, dispersions of which were prepared in aqueous and ethanol solutions under stirring or in an ultrasonic bath. Nalco alumina (particle size 8 nm, SSA of 550 ± 20 $m^2 g^{-1}$) was supplied as a 22 wt% dispersion in water.

Preparation of drug coated alumina particles

Stock solutions of naproxen in water and ethanol were prepared in concentrations of 0.05, 0.1, and 0.15 mol dm^{-3}. Naproxen dissolves directly in ethanol. To obtain solutions in water, the solids were first dissolved at a pH of about 11, and then the pH was slowly decreased to 7.2 by adding a few drops of hydrochloric acid. All solutions were freshly prepared and filtered through 0.22 μm Millipore membranes before use.

To study the adsorption of drugs, a known volume of colloidal dispersions of either Nalco or DGC alumina was first diluted either with water or with ethanol as needed. Then, desired volumes of the stock solutions of different concentrations of naproxen were added under stirring to reach a final volume of 100 cm^3. The adsorption experiments with DGC samples were carried out in aqueous and ethanol media, and with the Nalco material only in water.

To avoid aggregation of alumina nanoparticles, their concentration was kept at 0.05 wt% in all experiments, while the concentration of naproxen was varied from 5 x 10^{-5} to 0.15 mol dm^{-3}. The mixtures were equilibrated for 15 hours at room temperature, and the particles were then separated by centrifugation at 55,000 rpm for 3 hours. The resulting solids were dried in a vacuum oven at 100°C overnight and stored in a

desiccator before further analyses. To prevent the aggregation of particles by the described procedure, some of the dispersions were freeze-dried. The separated solids were redispersible in water by using an ultrasonic bath.

Experimental: Preparation of Ketoprofen Particles

All organic chemicals were reagent grade and were not further purified. Ketoprofen (MW: 270 g mol^{-1}) was obtained from Elan Pharmaceutical Technologies.

Preparation of drug coated alumina particles

The ketoprofen particles were prepared as described above for the naproxen particles.

Characterization of Particles

The presence of the drugs at the alumina surfaces was checked by attenuated total reflection infrared (FTIR-ATR) spectroscopy over the 4000-500 cm^{-1} range at a resolution of 4 cm^{-1}. The quantities of the adsorbed organics were assayed by the thermogravimetric analysis (TGA), carried out at temperatures ranging from 100 to 1000 °C, at the scan rate of 20 °C/min in air. The alumina nanoparticles coated with drugs were also examined by transmission electron microscopy (TEM).

Electrokinetic measurements of samples in water and ethanol were performed with a Zetapals instrument at a pH of 7.2 ± 0.2 in 1.10^{-3} mol dm^{-3} NaCl at 25°C. Each measurement was repeated ten times.

Results

Electrophoresis was used as a convenient technique to preliminary assess if there was any drug attachment to the alumina cores. Since both naproxen and ketoprofen have carboxyl groups, it was expected that the carboxyl groups would condense with the basic surface sites of the adsorbent.

First, the ζ-potentials of both alumina samples were evaluated as a function of the pH in a 10^{-3} mol dm^{-3} NaCl aqueous solutions, which yielded isoelectric points (IEP) between 9 and 9.5 (Figure 1), characteristic of colloidal alumina. G.A. Parks, *Chem. Revs.*, *65*:177 (1965). Most experiments were carried out at pH ~ 7 to have cores positively charged.

Figure 2 displays the electrokinetic data of alumina particles in the presence of different concentrations of naproxen and ketoprofen. In aqueous solutions the ζ-potential first becomes less positive and with further addition of the drugs, the sign is reversed to negative. The concentration of naproxen at which the reversal of charge takes place is ~ $5 \cdot 10^{-3}$ mol dm^{-3} for DGC and ~ $1.6 \cdot 10^{-2}$ mol dm^{-3} for the Nalco sample, while the charge of the DGC is reversed with ketoprofen at $1 \cdot 10^{-3}$ mol dm^{-3}. Interestingly, no charge reversal is observed in ethanol-containing dispersions. These results confirm that the drugs interact with alumina cores.

The adsorption data of the drugs on DGC alumina in water and in ethanol show the uptake of ketoprofen to be substantially higher than that of naproxen in both media (Figure 3). The saturation surface concentration of ketoprofen in aqueous media is at ~4.3 μmol m^{-2} and in ethanol at ~ 4.7 μmol m^{-2}, while the corresponding value of naproxen is ~2.3 μmol m^{-2} in both media. These calculations are based on the specific surface area information provided by the inorganic core manufacturer.

The infrared spectra of the cores, of naproxen, and of alumina coated particles with naproxen clearly show that the latter is not a simple combination of the individual ones (Figure 4). The Al-OH stretching vibration at 1020 cm^{-1} is quenched, while a new vibration at 1295 cm^{-1} appears on naproxen coated alumina particles (Figure 4 c). The C-H stretching vibration between 2900 and 3000 cm^{-1} and C=C vibration of the ring stretch adsorption at 1511 cm^{-1} are characteristic of naproxen. These data support the conclusion that a covalent bond is formed between the adsorbent and the drug molecules.

The electron micrograph in Fig. 5 illustrates the Nalco alumina cores and the same material coated with naproxen. The primary particle size is of the order of 10 nm,

while the alumina coated particles consist of small aggregates estimated to be 30 nm in size. Analogous electron micrographs were obtained with the DGC alumina systems.

Discussion

The uptake by weight of ketoprofen at saturation is more than twice that of naproxen, both in aqueous and alcohol media. Two properties of these molecules may account for the observed differences. First the molecular mass of ketoprofen (270 g mol^{-1}) is higher than that of naproxen (230 g mol^{-1}), although this difference is not sufficient to account for the much higher coating weight of the former. The other parameter to consider is the shape of the molecules. Since the carboxyl groups are oriented towards the surface of the inorganic cores, a larger number of ketoprofen molecules can be accommodated at saturation than of naproxen molecules, due to the steric interference of their naphthalene rings. Indeed, at saturation amount the area per molecule of adsorbed naproxen is 71 $Å^2$ and 37 $Å^2$ for ketoprofen at the DGC surface.

In addition, the results indicate a difference in the electrokinetic behavior of the same coated particles in water and alcohol. As indicated above, both drug species are adsorbed by interaction of carboxyl groups with basic alumina sites on the cores, which is consistent with earlier electrokinetic data on amino acids bound to metal (hydrous) oxides. Kumanomido et al., *J. Colloid Interface. Sci., 66:*183 (1978). However, since charge reversal to negative values takes place, some carboxyl groups of the adsorbed molecules must still be dissociated.

Furthermore, while negative ζ-potential of particles coated with naproxen remains constant above a certain concentration of the drug, it decreases with increasing amount of the added ketoprofen. This difference may also be explained in terms of surface conformation of the two kinds of molecules. On crowding of adsorbed ketoprofen molecules, the dissociation of neighboring carboxyl groups is depressed due to electrostatic repulsion, leading to a lower potential.

Finally, the fact that no charge reversal with both drugs takes place in alcohol solution indicates that ethanol prevents the dissociation of the surface oriented

carboxyls.

Example 2

The purpose of this example was to prepare nanoparticulate compositions of inorganic cores having naproxen coated thereon.

Materials

All organic chemicals were reagent grade and were not further purified. Naproxen and Ludox CL® (12 nm alumina coated silica) were obtained from Aldrich Chemicals. A1umin (8 nm) was supplied by Nalco Chemical Company, and colloidal silica (20 nm) in methanol was obtained from Nissan Company.

Experimental

To adsorb naproxen, stock solutions of 0.1 mol dm^{-3} water/naproxen and 0.1 mol dm^{-3} methanol/naproxen were prepared. A known volume of the colloidal suspension of silica or alumina was taken and mixed with a given volume of the stock solution of naproxen. A sufficient volume of water or methanol was added to bring the total volume to 40 cm^3. By varying the concentrations of naproxen and silica or alumina in different solvents, various systems were obtained.

To prepare samples for attenuated total reflection infra-red (ATR-FTIR) analyses, silica or alumina nanoparticles were separated from the solvent by centrifugation at 55,000 rpm for 2 hours and then dried in a oven overnight. The ATR-FTIR spectra were taken over 4000-500 cm^{-1} range at a resolution of 4 cm^{-1}.

A summary of the preparation conditions for the formation of nanoparticulate silica (Ludox CL®) coated with naproxene is given in Table 1.

TABLE 1			
Conditions for the Preparation of Ludox CL silica –Naproxen/Water- Systems			
Concentration of silica (wt%)	**Concentration of naproxen (mol dm^{-3})**	**pH**	**Observation**
10	0.1		Gel
10	0.05		Gel
10	0.01		Gel
2	0.1	9.5	Turbid and phase separation
2	0.05	9.2	Same as above
2	0.01	9.4	Same as above
2	0.1	9	Turbid
2	0.01	8.55	Turbid
1	0.1	9	Turbid
0.5	0.01	9.82	Turbid
0.5	0.01	8.08	Turbid

Because naproxen does not dissolve in aqueous medium even at pH of 10, and that the isoelectric point of the Ludox CL® is between 9 and 9.5, a non-aqueous solvent for naproxen is required. To identify a suitable solvent, the solubility of naproxen in organic solvents was tested, as detailed below in Table 2.

TABLE 2	
Test of Solubility of Naproxen in Organic Solvents	
Solvent + Naproxen	**Observation**
Isopropanol	Insoluble
Ethyl lactate	Insoluble
propylene glycol monomethyl ether acetate (PMA)	Insoluble
Tetrahydrofuran (THF)	Insoluble
Hexane	Insoluble
Decane	Insoluble
Methanol	Dissolved
Ethanol	Dissolved

Because naproxen dissolved well in ethanol and methanol, solutions of naproxen in these two solvents were prepared, as described below, to make naproxen-coated silica

nanoparticulate particles.

TABLE 3		
Conditions for the Preparation of SiO_2 (Ludox CL®) silica –Naproxen/Ethanol- Systems		
Concentration of Ludox CL® (wt%)	Concentration of naproxen (mol dm^{-3})	Observation
2	0.1	Turbid
2	0.05	Turbid
2	0.01	Turbid
1	0.01	Turbid
0.5	0.01	Turbid
0.25	0.01	Turbid

TABLE 4		
Conditions for the Preparation of SiO_2 (Nissan in methanol) silica – Naproxen/Methanol- Systems		
Concentration of Nissan silica (wt%)	Concentration of naproxen (mol dm^{-3})	Observation
2	0.1	Clear
2	0.01	Clear

Following is a summary of the conditions for preparation of alumina coated naproxen particles in water and methanol.

TABLE 5			
Conditions for the Preparation of Al_2O_3 –Naproxen/Water- Systems			
Concentration of alumina (wt%)	Concentration of naproxen (mol dm^{-3})	pH	Observation
2	0.0025	8.5	Turbid

TABLE 6		
Conditions for the Preparation of Al_2O_3 –Naproxen/Methanol- Systems		
Concentration of alumina (wt%)	Concentration of naproxen (mol dm^{-3})	Observation
2	0.05	Clear
2	0.01	Clear

The formulations described above were then subjected to attenuated total

reflection infrared (FTIR-ATR) spectroscopy over the 4000-500 cm^{-1} range at a resolution of 4 cm^{-1} to determine the presence of naproxen at the surface of the alumina and silica cores.

Specifically, Figure 6 shows FTIR spectra of: (a) naproxen; (b) Ludox CL® (2 wt%) + naproxen (0.01 mol. dm^{-3} at pH = 8.55 in water; and (c) Ludox CL® silica; Figure 7 shows FTIR spectra of: (a) naproxen; (b) Ludox CL® (2 wt%) + naproxen (0.01 mol. dm^{-3} in ethanol; and (c) Ludox CL® silica; Figure 8 shows FTIR spectra of: (a) naproxen; (b) Nissan silica (2 wt%) + naproxen (0.01 mol. dm^{-3} in methanol; and (c) Nissan silica; Figure 9 shows FTIR spectra of: (a) naproxen; (b) Nalco alumina (0.1 wt%) + naproxen (0.0025 mol. dm^{-3} in water; and (c) alumina Nalco; and Figure 10 shows FTIR spectra of: (a) naproxen; (b) Nalco alumina (2 wt%) + naproxen (0.05 mol. dm^{-3} in methanol; and (c) alumina Nalco.

Despite the numerous adsorption systems studied, there was no evidence for interaction between the naproxen and the silica or alumina core surfaces. This finding is caused by the adverse solubility and surface charge characteristics of the reactants.

Example 3

The purpose of this example was to successfully prepare nanoparticulate compositions of inorganic cores having naproxen coated thereon.

Experimental

Materials

All organic chemicals were reagent grade and were not further purified. Naproxen was obtained from Aldrich Chemicals. Commercially available alumina nanoparticles, Degussa C, γ-Al_2O_3 (particle size 13 nm, specific surface area (SSA) of 100 ± 10 m^2g^{-1}) and Nalco (particle size ~ 8 nm, SSA of 550 ± 20 $m^2 g^{-1}$) were supplied by the Degussa and Nalco Chemical Companies, respectively. These particles were used as inorganic cores.

Preparation of Naproxen Coated Alumina Particles

Stocks solutions of naproxen in water and ethanol were prepared in concentrations of 0.05, 0.1, and 0.2 mol dm^{-3}. The drug dissolved directly in ethanol. To prepare solutions of naproxen in water, the pH was first raised to 11 and then slowly decreased to 7.2 by adding a few drops of hydrochloric acid. All solutions were freshly prepared and filtered through 0.22 μm Millipore membranes before use.

To adsorb naproxen on alumina surfaces, a known volume of the colloidal dispersion of Nalco or Degussa C alumina powders was mixed under stirring with a given volume of solvents (water or ethanol). Then the volumes of the stock solutions containing different concentrations of naproxen were added under stirring to reach a final volume of 100 cm^3. To avoid aggregation of alumina nanoparticles, the concentration was kept at 0.05 wt% in all experiments, while the concentration of naproxen was varied from 5 x 10^{-5} to 0.1 mol dm^{-3}. The mixtures were equilibrated for 15 hours at room temperature.

The obtained particles were separated from water or ethanol by centrifugation at 55,000 rpm for 3 hours. The resulting solids were dried in a vacuum oven at 100°C overnight and stored in a desiccator before further analyses. This process produces cakes, which were ground before some of the analyses. To prevent the aggregation of particles by the described procedure, some of the dispersions were freeze-dried.

Characterization of Nanoparticulate Alumina Coated with Naproxen

The size of obtained naproxen coated alumina nanoparticles was examined by transmission electron microscopy (TEM). The presence of the naproxen at the alumina surfaces was checked by attenuated total reflection infrared (ATR-FTIR) spectroscopy over 4000-500 cm^{-1} range at a resolution of 4 cm^{-1}. The TGA was carried out at temperatures ranging from 100 to 1000°C at the scan rate of 20 °C/min in air, to determine the quantities of the adsorbed naproxen on alumina.

Electrokinetic measurements of naproxen coated alumina nanoparticles in water and ethanol were performed with a ZetaPALS instrument at a pH of 7.2 ± 0.2 in 1 x 10^{-3} mol dm^{-3} NaCl at 25°C. Each measurement was repeated ten times.

A summary of the preparation conditions in the formation of nanoparticulate alumina coated with naproxen is given in Table 7.

TABLE 7

Concentration of Naproxen Used in All Formulations

Concentration of Naproxen (mol dm^{-3})	0	0.001	0.0025	0.005	0.01	0.025	0.1

Degussa C alumina powder in water and ethanol, and Nalco alumina in water, was used for the naproxen formulations. The concentration of alumina was kept to 0.05 wt.% and the pH of the aqueous solutions was 7.2 ± 0.1.

Figure 11 shows the ζ-potentials of both alumina cores, Nalco alumina (O) and Degussa C alumina (□), in 1 x 10^{-3} mol dm^{-3} aqueous NaCl solution, as a function of the pH. The isoelectric point (IEP) of both samples is between 9 and 9.5, which is characteristic of colloidal alumina. In most experiments pH ~ 7 was chosen to have cores positively charged.

Figure 12 shows the ζ -potentials of both alumina cores, Nalco alumina (O) and Degussa C alumina (□), in 1 x 10^{-3} mol dm^{-3} aqueous NaCl solution at a pH of 7.2 ± 0.1, and of Degussa C alumina in ethanol (Δ) as a function of the concentration of added naproxen. Figure 12 thus gives the electrokinetic data of alumina particles in the presence of different concentrations of naproxen. The figure shows that with the addition of naproxen, the ζ-potential first becomes less positive but with an increasing concentration of the drug in aqueous dispersions the sign of the charge is reversed to negative. The concentration at which the reversal of charge takes place is ~ 5.10^{-3} mol dm^{-3} of naproxen for Degussa C and ~$1.6 \cdot 10^{-2}$ mol dm^{-3} of naproxen for the Nalco sample. The effect is much more pronounced with the larger cores (Degussa C). Interestingly, no charge reversal is observed in the ethanol-containing dispersions. These results independently confirm that naproxen interacts with the alumina cores.

Figure 13 shows the adsorption isotherms of naproxen on Degussa C alumina in water (□) at a pH of 7.2 ± 0.1 and in ethanol (Δ). The concentration of alumina in all

samples was 0.05 wt.%. While at the lower drug concentration the adsorption is more efficient in alcohol, on saturation the same amount of adsorbed naproxen is found, which corresponds to 2.3 μmol m^2 (*i.e.* 5.2 wt%). These data are based on the SSA supplied by the manufacturer.

Figure 14 shows the FTIR spectra of Degussa C alumina (a), naproxen (b), and Degussa C alumina coated with naproxen in ethanol (c). The ATR-FTIR spectra confirm the presence of naproxen at the Degussa alumina surfaces. The C-H stretching vibration between 2900 and 3000 cm^{-1} and C=C vibration of ring stretch adsorption at 1511 cm^{-1} are characteristic of naproxen. Furthermore, the Al-OH stretching vibration at 1020 cm^{-1} was quenched and the appearance of a new vibration at 1295 cm^{-1} was observed on naproxen coated alumina particles (Fig. 4c). The quenching of the Al-OH stretching at 1020 cm^{-1} (Fig. 4a) and the appearance of a new vibration at 1295 cm^{-1} (Figure 4c) can probably be attributed to the formation of a metallic complex between naproxen and alumina.

The Nalco alumina sample interacted with naproxen as displayed in Fig. 15, which shows the same effects as the Degussa sample. Specifically, Fig. 15 shows the FTIR spectra of Nalco alumina (a); naproxen (b); and Nalco alumina coated with naproxen in water (c).

Figure 16 shows transmission electron micrographs of the Nalco alumina core (a) and of Nalco alumina core coated with naproxen (b). The primary particle size is about 10 nm (Figure 16a). The alumina coated with naproxen composition comprises particles consisting of small aggregates estimated to be about 30 nm in size.

Corresponding electron micrographs of the Degussa alumina cores and of the same naproxen-coated cores are shown in Fig. 17. It is apparent that on drying aggregation of both cores and coated particles takes place, but modified particles are still nanosized. However, such aggregation may not occur in the dispersions before drying.

These experimental results show the successful preparation of nanoparticulate sized drug coated inorganic cores, without significant particle aggregation. Moreover,

any initial aggregates can be peptized by ultrasonic treatment, producing stable dispersions. The nanoparticulate particle sizes produced in this experiment were about 8 and 13 nm, although this size can be varied by varying the size of the inorganic core.

The experimental results also show that the tested compositions allow for adsorption of the drug to proceed through a bond formation between the inorganic core and the drug coating.

Example 4

The purpose of this example was to successfully prepare uniform nanoparticulate drug particles by coating nanoparticulate alumina cores with ketoprofen.

Experimental

Materials

All organic chemicals were reagent grade and were not further purified. Ketoprofen was provided by Elan Pharmaceutical Technologies. Commercially available alumina nanoparticles, Degussa C, γ-Al_2O_3, (particle size 13 nm, specific surface area (SSA) of 100 ± 10 m^2g^{-1}) were supplied by the Degussa Company. Colloidal silica, MP4540 (particle size 0.45 μm), was purchased from Nissan Company.

Preparation of ketoprofen coated alumina particles

Stock solutions of ketoprofen in water and ethanol were prepared in concentrations of 0.05, 0.1, and 0.15 mol dm^{-3}. Ketoprofen dissolves directly in ethanol. To prepare solutions of ketoprofen in water, the pH was first raised to 12 and then slowly decreased to 7.2 by adding a few drops of hydrochloric acid. All solutions were freshly prepared and filtered through 0.22 μm Millipore membranes before use.

To adsorb ketoprofen on alumina surfaces, a known volume of Degussa C alumina powder was mixed under stirring with a given volume of solvents (water or ethanol). Afterwards desired volumes of stock solutions, containing different concentrations of ketoprofen, were added under stirring to reach a final volume of 100 cm^3. To avoid

aggregation of alumina nanoparticles, the concentration was kept at 0.05 wt% in all experiments, while the concentration of ketoprofen was varied from 5×10^{-5} to 0.15 mol dm^{-3}. The mixtures were equilibrated for 15 hours at room temperature. The obtained particles were separated from water or ethanol by centrifugation at 55,000 rpm for 3 hours. The resulting solids were dried in a vacuum oven at 100°C overnight and stored in a desiccator before further analyses. The produced cakes were ground before some of the analyses. To prevent the aggregation of particles by the described procedure, some dispersions were freeze-dried.

Precipitation of cyclosporin on silica or hematite

Freshly prepared stock solutions of cyclosporin in ethanol in concentrations of 0.03 and 0.01 mol dm^{-3}, respectively, were filtered through 0.22 μm Millipore membranes before use. In a typical experiment, volumes of stock solutions containing different concentrations of cyclosporin were added to silica or hematite dispersions (0.1 wt%) in ethanol under stirring to reach a final volume of 50 cm^3. After one minute of agitation, distilled water (a nonsolvent for cyclosporin) was admixed in amounts either to reach just below or just above the precipitation onset of the drug.

Characterization of Materials

Alumina nanoparticles coated with ketoprofen were examined by TEM. The presence of the ketoprofen on alumina surfaces was evaluated by attenuated total reflection infrared (ATR-FTIR) spectroscopy over the 4000-500 cm^{-1} range at a resolution of 4 cm^{-1}. To determine the amounts of the adsorbed ketoprofen on alumina the TGA was carried out at temperatures ranging from 100 to 1000°C at the scan rate of 20°C/min. in air.

Electrokinetic measurements of ketoprofen-coated alumina nanoparticles in water and ethanol were performed with a Zetapals instrument at a pH of 7.2 ± 0.2 in 1×10^{-3} mol dm^{-3} NaCl at 25°C. Each measurement was repeated ten times.

Results of Ketoprofen Coated Alumina Nanoparticles

A summary of the preparation conditions in the formation of nanoparticulate alumina (Degussa C in water and ethanol) coated with ketoprofen is given in Table 8.

TABLE 8

Concentration of Ketoprofen Used in All Formulations

Concentration of Naproxen (mol dm^{-3})	0	0.001	0.0025	0.005	0.01	0.025	0.1

The concentration of alumina was kept at 0.05 wt.% and the pH of the aqueous solutions was 7.2 ± 0.1.

Electrokinetic data

Figure 18 shows the ζ-potentials of Degussa C alumina (□) in 1 $x10^{-3}$ mol dm^{-3} NaCl aqueous solution at pH = 7.2 ± 0.1, and of Degussa C alumina in ethanol (Δ) as a function of the concentration of added ketoprofen. Thus, Fig. 18 shows the electrokinetic data of alumina particles, in aqueous dispersions, in the presence of different concentrations of ketoprofen. The figure demonstrates that with the addition of ketoprofen, the ζ-potential first becomes less positive, and with an increasing concentration of ketoprofen the sign of the charge is reversed to negative. The concentration at which the reversal of charge takes place is less than 1.10^{-3} mol dm^{-3} of ketoprofen for Degussa C. Interestingly, no charge reversal is observed in ethanol-containing dispersions and, in this case, the ζ-potential does not show any significant variation. There may be several reasons for the alcohol effect, including a different isoelectric point, the significance of the pH, and a possible change in the conformation of the adsorbed drug molecules. These results confirm that ketoprofen interacts with the alumina cores.

Adsorption of ketoprofen on alumina cores

Figure 19 shows the adsorption data of ketoprofen on Degussa C alumina in water and in ethanol. The adsorption is more efficient in alcohol, yielding somewhat

larger amounts of deposited drug. The amounts on saturation are ~ 4.7 μmol m^2 (*i.e.*,12 wt%) for adsorption in ethanol and 4.3 μmol m^2 (*i.e.*, 10.8 wt%) for adsorption in water. These data are based on the SSA supplied by the inorganic core manufacturer, and corresponds to an average molecular area per ketoprofen molecule of 37 $Å^2$. It is interesting to note that ketoprofen adsorbs twice as much as naproxen on the same cores (~ 5.2 wt%).

Infrared Spectra

The ATR-FTIR spectra (Figure 20) confirm the presence of ketoprofen at the Degussa C alumina surfaces. The C-H stretching vibration between 2900 and 3000 cm^{-1} are characteristic of ketoprofen.

Transmission Electron Microscopy of Degussa C alumina coated with ketoprofen

Electron micrographs of Degussa alumina cores are shown in Figure 14a. Corresponding electron micrographs of the Degussa alumina cores coated with ketoprofen are shown in Fig. 21. It is apparent that on drying aggregation of coated particles takes place, but modified particles are still nanosized. However, such aggregates may not be present in dispersions before drying.

Example 5

The purpose of this example was to prepare uniform nanoparticulate drug particles by coating nanoparticulate silica or hematite cores with cyclosporine.

Experimental

Materials

All organic chemicals were reagent grade and were not further purified. Cyclosporine was provided by Elan Pharmaceutical Technologies. Colloidal silica, MP4540 (particle size 0.45 μm), was purchased from Nissan Company. Hematite (α-Fe_2O_3) was synthesized and used as inorganic cores.

The compositions were prepared as in Example 4.

Precipitation of cyclosporin on silica or hematite

Precipitation of cyclosporin on silica

Figure 22 shows the SEM picture of the original monodispersed silica particles of 0.45 μm in diameter. In these experiments, relatively large silica cores were used for practical reasons to explore possible deposition of the drug. These particles are much more easily manipulated than nanoparticulate sized particles. Figure 23 shows the SEM picture of the same silica after equilibration with cyclosporin, and Figure 24 shows the infrared spectra of this system, which indicate no presence of the drug. Similarly, no uptake could be detected by TGA.

Precipitation of cyclosporin on hematite

Figure 25 displays the SEM of the original hematite (α-Fe_2O_3) which are ellipsoidal (~ 0.7 μm long and 0.1 μm wide), while Figure 26 is the SEM of the same hematite after the equilibration with cyclosporin. No change in particles could be seen, although they appear to adhere to each other, possibly enmeshed by cyclosporin molecules.

Example 6

The purpose of this example was to determine the effect on the adsorption of naproxen and ketoprofen to a modified surface of nanoparticulate silica particles.

Experimental

Materials

All organic chemicals were reagent grade and were not further purified. Ketoprofen was provided by Elan Pharmaceutical Technologies. Naproxen was obtained from Aldrich Chemicals. IPA-ST-S (particle size 12 nm) in isopropanol was purchased from Nissan Company. Ludox CL® (particle size 22 nm, SSA of 130 ± 10 m^2g~) and Ludox AM® (particle size 12 nm specific surface area, SSA of 220 ± 10

m^2g^{-1}) were obtained from Dupont Company. The coupling agents N-phenylaminopropyltrimethoxysilane, 3-aminopropylmethyldiethoxysilane, and methacryloxypropyltrimethoxysilane were obtained from United Chemical Technologies.

Experimental

Table 9 lists the different kinds of silica particles and coupling agents used in the experiment.

Silica surface modification

In all cases 5 g of a coupling agent was slowly added to 100 cm^3 of the colloidal silica dispersion and the systems were equilibrated for one week at room temperature. Modified silica was then centrifuged for three hours at 55,000 rpm and the resulting gel was redispersed in the original solvent.

TABLE 9

Silica	Solvent	Coupling agent	Observation	Reaction with Naproxen	Reaction with Ketoprofen
Ludox-AM®	water	N-phenylaminopropyl trimethoxysilane	stable	no	Yes
Ludox-AM®	water	3-aminopropylmethyl diethoxysilane	gel	no	no
Ludox-CL®	water	N-phenylaminopropyl trimethoxysilane	stable	no	no
IPA-ST-S®	Iso-propanol	Methacryloxypropyl-trimethoxysilane	stable	no	no
IPA-ST-S®	iso-propnol	N-phenylaminopropyl trimethoxysilane	stable	no	no

The results from infrared (Figure 27, the C-H stretching vibration between 2900 and 3000 cm^{-1} is characteristic of ketoprofen) and TGA measurements show that the modified Ludox AM® with N-phenylaminopropyltrimetoxysilane interacts with ketoprofen, while ketoprofen does not interact with the untreated silica.

* * * *

It will be apparent to those skilled in the art that various modifications and variations can be made in the methods of the present invention without departing from the spirit or scope of the invention. Thus, it is intended that the present invention cover the modifications and variations of this invention provided they come within the scope of the appended claims and their equivalents.

We claim:

1. A stable nanoparticulate composition comprising:

(a) at least one type of nanoparticulate inorganic core having a particle size of less than about 1 micron; and

(b) at least one active agent adsorbed on the surface thereof.

2. The composition of claim 1, wherein the at least one type of nanoparticulate inorganic core is present in an amount of about 0.1% to about 99.9 (w/w).

3. The composition of claim 1, wherein the at least one type of inorganic core is selected from the group consisting of metal oxides, metal hydroxides, metal hydrous oxides, metal sulfides, metal sulfates, metal carbonates, and metal phosphates.

4. The composition of claim 3, wherein the at least one type of inorganic core is selected from the group consisting of titanium dioxide, zinc oxide, chromium oxide, chromium hydroxide, silver halides, cadmium oxide, cadmium sulfide, zinc sulfide, copper oxide, barium sulfate, cerium oxide, and cobalt oxide.

5. The composition of claim 1, wherein the at least one type of inorganic core is selected from the group consisting of alumina, silica, and hematite.

6. The composition of claim 1, further comprising at least two different types of inorganic cores, wherein the at least two inorganic cores are made of different materials.

7. The composition of claim 1, wherein the at least one inorganic core has a particle size selected from the group consisting of less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm,

less than about 300 nm, less than about 200 nm, less than about 100 nm, less than about 75 nm, less than about 50 nm, less than about 25 nm, less than about 15 nm, less than about 10 nm, and less than about 5 nm.

8. The composition of claim 1, further comprising an organic core of at least one type having at least two different particle sizes.

9. The composition of claim 8, wherein the at least two different particle sizes are selected from the group consisting of less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 200 nm, less than about 100 nm, less than about 75 nm, less than about 50 nm, less than about 25 nm, less than about 15 nm, less than about 10 nm, and less than about 5 nm.

10. The composition of claim 1, wherein the at least one active agent is present in an amount of about 0.001% to about 99.9% (w/w).

11. The composition of claim 1, wherein the at least one active agent is a drug selected from the group consisting of proteins, peptides, nutraceuticals, anti-obesity agents, corticosteroids, elastase inhibitors, analgesics, anti-fungals, oncology therapies, anti-emetics, analgesics, cardiovascular agents, anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, antibiotics, anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytics, sedatives (hypnotics and neuroleptics), astringents, beta-adrenoceptor blocking agents, blood products and substitutes, cardiac inotropic agents, contrast media, cough suppressants (expectorants and mucolytics), diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics (antiparkinsonian agents), haemostatics, immunological agents, lipid

regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin and biphosphonates, prostaglandins, radio- pharmaceuticals, sex hormones (including steroids), anti-allergic agents, stimulants and anoretics, sympathomimetics, thyroid agents, vasodilators, xanthines, bronchodilators, cystic-fibrosis therapies, asthma therapies, emphysema therapies, respiratory distress syndrome therapies, chronic bronchitis therapies, chronic obstructive pulmonary disease therapies, organ-transplant rejection therapies, therapies for tuberculosis and other infections of the lung, and respiratory illness therapies associated with acquired immune deficiency syndrome.

12. The composition of claim 1, wherein the at least one active agent is selected from the group consisting of a sunscreen agent, a cosmetic, an acne medication, an anti-wrinkle drug, nail polish, a moisturizer, a fragrance, a deodorant, a hair dye, a hair spray, a hair cosmetic, a hair cleanser, and a depilatory.

13. The composition of claim 1, wherein the at least one active agent is selected from the group consisting of a pesticide, insecticide, fertilizer, acaricide, germicide, plant growth regulating agent, herbicide, photosynthesis inhibitor, pigment inhibitor, growth inhibitor, amino acid synthesis inhibitor, lipid biosynthesis inhibitor, cell wall biosynthesis inhibitor, and a rapid cell membrane disruptor.

14. The composition of claim 1, comprising at least two different active agents.

15. A nanoparticulate composition comprising:

(a) at least one nanoparticulate inorganic core having a particle size of less than about 1000 nm;

(b) at least one active agent; and

(c) at least one coupling agent that links the active agent molecules to the inorganic cores.

16. The composition of claim 15, wherein the at least one coupling agent is selected from the group consisting of amino silanes and caroxy silanes.

17. The composition of claim 16, wherein the at least one coupling agent is selected from the group consisting of N-phenylaminopropyltrimethoxysilane, 3-aminopropylmethyldiethoxysilane, and methacryloxypropyltrimethoxysilane.

18. The composition of claim 15, wherein the at least one type of nanoparticulate inorganic core is present in an amount of about 0.1% to about 99.9% (w/w).

19. The composition of claim 15, wherein the at least one type of inorganic core is selected from the group consisting of metal oxides, metal hydroxides, metal hydrous oxides, metal sulfides, metal sulfates, metal carbonates, and metal phosphates.

20. The composition of claim 19, wherein the at least one type of inorganic core is selected from the group consisting of titanium dioxide, zinc oxide, chromium oxide, chromium hydroxide, silver halides, cadmium oxide, cadmium sulfide, zinc sulfide, copper oxide, barium sulfate, cerium oxide, and cobalt oxide.

21. The composition of claim 19, wherein the at least one type of inorganic core is selected from the group consisting of alumina, silica, and hematite.

22. The composition of claim 15, further comprising at least two different types of inorganic cores, wherein the at least two inorganic cores are made of different materials.

23. The composition of claim 15, wherein the at least one inorganic core has a particle size selected from the group consisting of less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 200 nm, less than about 100 nm, less than about 75 nm, less than about 50 nm, less than about 25 nm, less than about 15 nm, less than about 10 nm, and less than about 5 nm.

24. The composition of claim 15, further comprising an organic core of at least one type having at least two different particle sizes.

25. The composition of claim 25, wherein the at least two different particle sizes are selected from the group consisting of less than about 800 nm, less than about 700 nm, less than about 600 nm, less than about 500 nm, less than about 400 nm, less than about 300 nm, less than about 200 nm, less than about 100 nm, less than about 75 nm, less than about 50 nm, less than about 25 nm, less than about 15 nm, less than about 10 nm, and less than about 5 nm.

26. The composition of claim 15, wherein the at least one active agent is present in an amount of about 0.001% to about 99.9% (w/w).

27. The composition of claim 15, wherein the at least one active agent is a drug selected from the group consisting of proteins, peptides, nutriceuticals, anti-obesity agents, corticosteroids, elastase inhibitors, analgesics, anti-fungals, oncology therapies, anti-emetics, analgesics, cardiovascular agents, anti-inflammatory agents, anthelmintics, anti-arrhythmic agents, antibiotics (including penicillins), anticoagulants, antidepressants, antidiabetic agents, antiepileptics, antihistamines, antihypertensive agents, antimuscarinic agents, antimycobacterial agents, antineoplastic agents, immunosuppressants, antithyroid agents, antiviral agents, anxiolytic sedatives (hypnotics and neuroleptics), astringents, beta-adrenoceptor blocking agents, blood

products and substitutes, cardiac inotropic agents, contrast media, cough suppressants (expectorants and mucolytics), diagnostic agents, diagnostic imaging agents, diuretics, dopaminergics (antiparkinsonian agents), haemostatics, immunological agents, lipid regulating agents, muscle relaxants, parasympathomimetics, parathyroid calcitonin and biphosphonates, prostaglandins, radio- pharmaceuticals, sex hormones (including steroids), anti-allergic agents, stimulants and anoretics, sympathomimetics, thyroid agents, vasodilators, xanthines, bronchodilators, cystic-fibrosis therapies, asthma therapies, emphysema therapies, respiratory distress syndrome therapies, chronic bronchitis therapies, chronic obstructive pulmonary disease therapies, organ-transplant rejection therapies, therapies for tuberculosis and other infections of the lung, and respiratory illness therapies associated with acquired immune deficiency syndrome.

28. The composition of claim 15, wherein the at least one active agent is selected from the group consisting of a sunscreen agent, a cosmetic, an acne medication, an anti-wrinkle drug, nail polish, a moisturizer, a fragrance, a deodorant, a hair dye, a hair spray, a hair cosmetic, a hair cleanser, and a depilatory.

29. The composition of claim 15, wherein the at least one active agent is selected from the group consisting of a pesticide, insecticide, fertilizer, acaricide, germicide, plant growth regulating agent, herbicide, photosynthesis inhibitor, pigment inhibitor, growth inhibitor, amino acid synthesis inhibitor, lipid biosynthesis inhibitor, cell wall biosynthesis inhibitor, and a rapid cell membrane disruptor.

30. The composition of claim 15, comprising at least two different active agents.

31. A method of making a nanoparticulate composition comprising mixing at least one solubilized active agent with at least one nanoparticulate inorganic core for a

time and under conditions to allow adsorption of the active agent to the inorganic core, wherein the inorganic core has a particle size of less than about 1 micron.

32. A method of making a nanoparticulate composition comprising mixing at least one solubilized active agent, at least one coupling agent, and at least one nanoparticulate inorganic core for a time and under conditions to allow binding between the active agent-coupling agent-inorganic core, wherein the inorganic core has a particle size of less than about 1 micron.

33. A method of treating a patient in need with a nanoparticulate composition comprising administering to a patient in need a therapeutically effective amount of a nanoparticulate composition, wherein said composition comprises:

(a) at least one type of nanoparticulate inorganic core having a particle size of less than about 1 micron; and

(b) at least one active agent adsorbed on the surface of the inorganic core.

34. A method of treating a patient in need with a nanoparticulate composition comprising administering to a patient in need a therapeutically effective amount of a nanoparticulate composition, wherein said composition comprises:

(a) at least one type of nanoparticulate inorganic core having a particle size of less than about 1 micron;

(b) at least one active agent adsorbed on the surface of the inorganic core; and

(c) a coupling agent that links the active agent molecules to the inorganic cores.

FIGURE 1

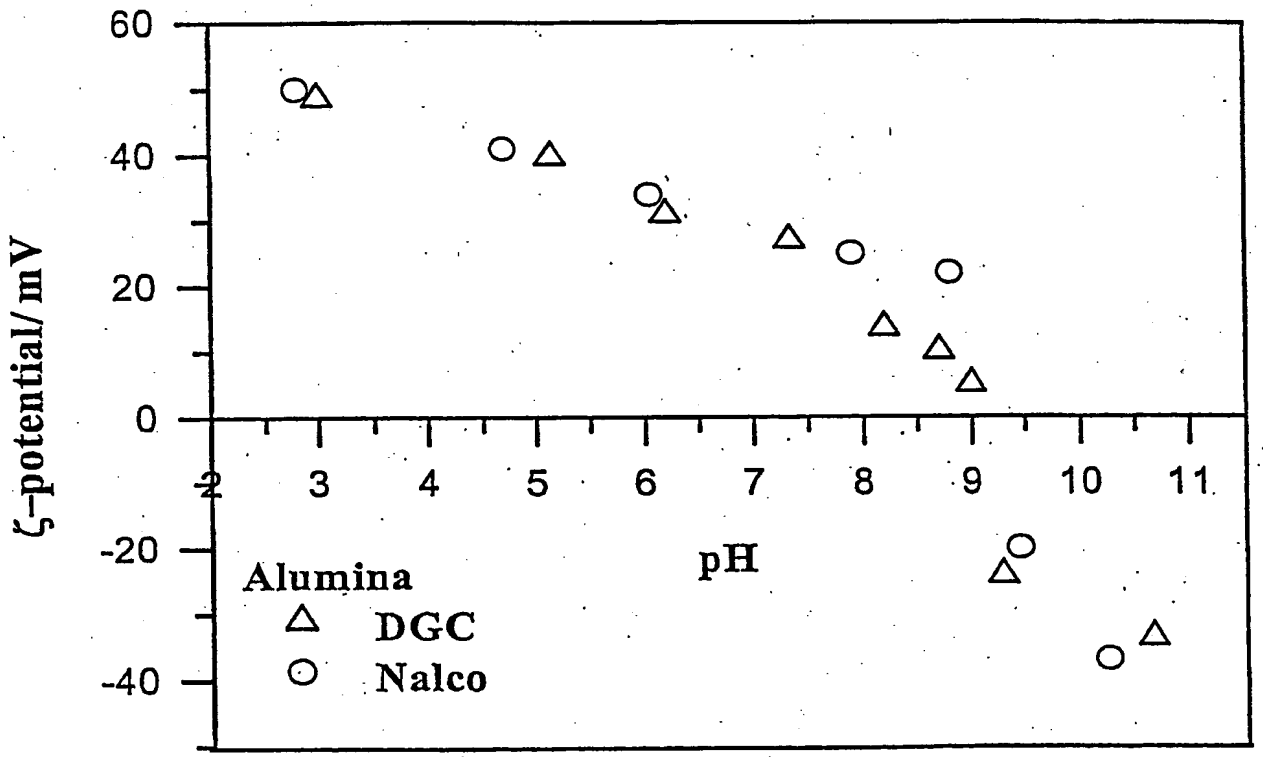

60
40
20
0
-20
-40
2
3
4
5
6
7
8
9
10
11
ζ-potential/ mV
pH
Alumina
DGC
Nalco

FIGURE 2

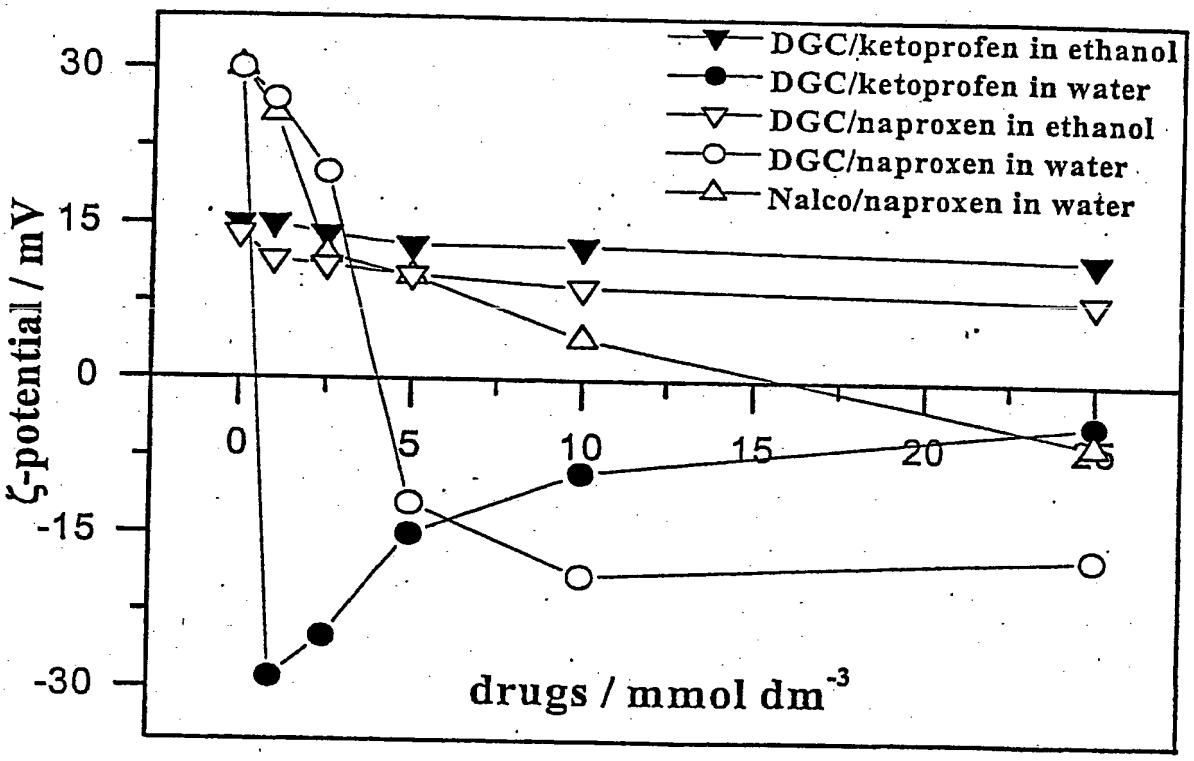

DGC/ketoprofen in ethanol
DGC/ketoprofen in water
DGC/naproxen in ethanol
DGC/naproxen in water
Nalco/naproxen in water
30
15
0
-15
-30
ζ-potential / mV
0
5
10
15
20
25
drugs / mmol dm-3

FIGURE 3

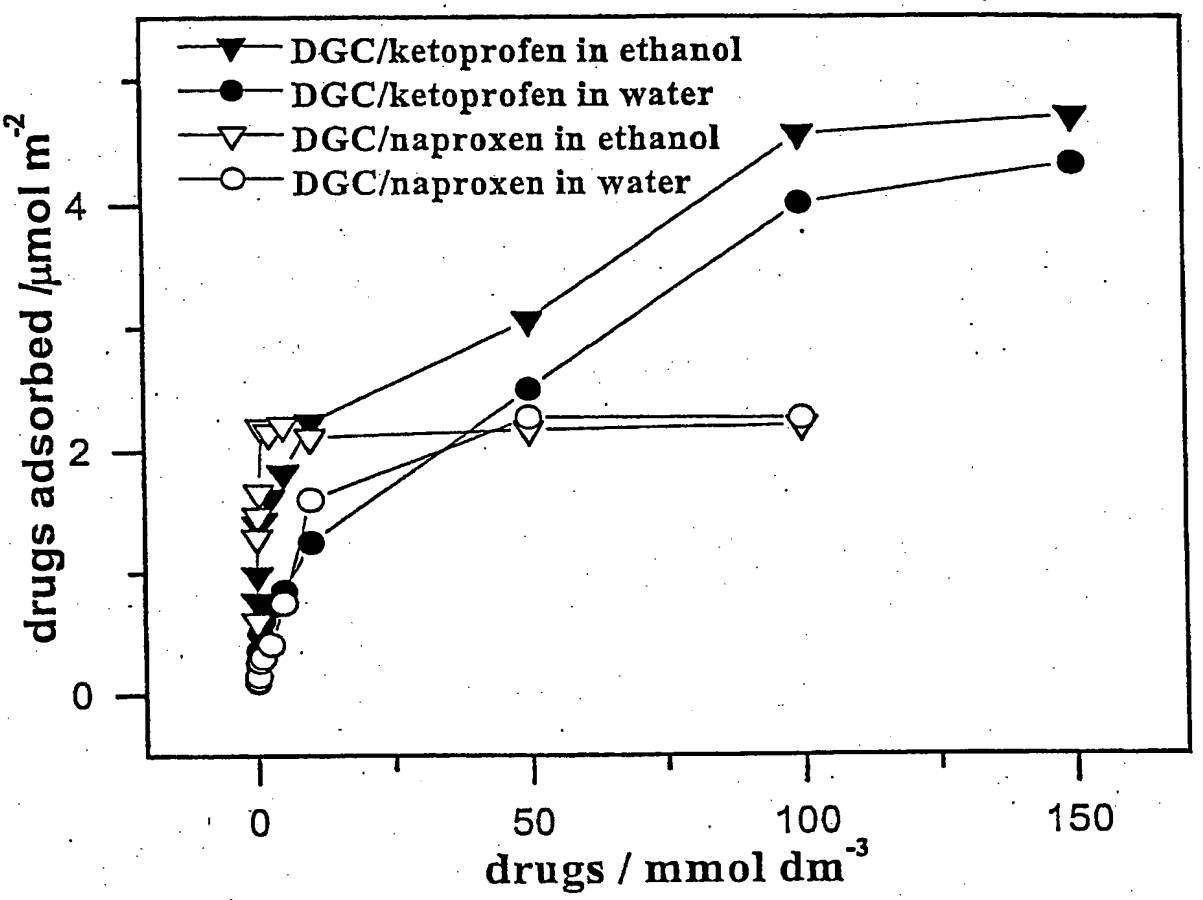

DGC/ketoprofen in ethanol
DGC/ketoprofen in water
DGC/naproxen in ethanol
DGC/naproxen in water
drugs adsorbed /μmol m-2
0
2
4
0
50
100
150
drugs / mmol dm-3

FIGURE 4

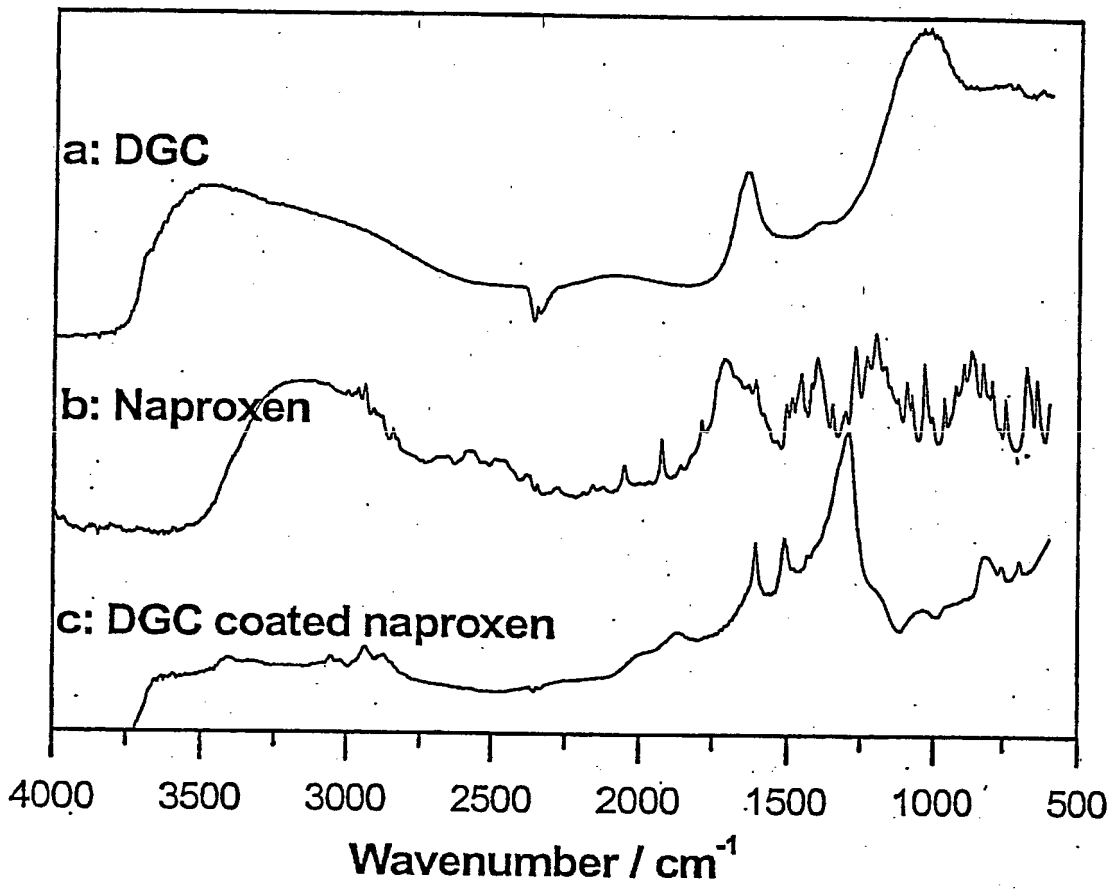


FIGURE 5

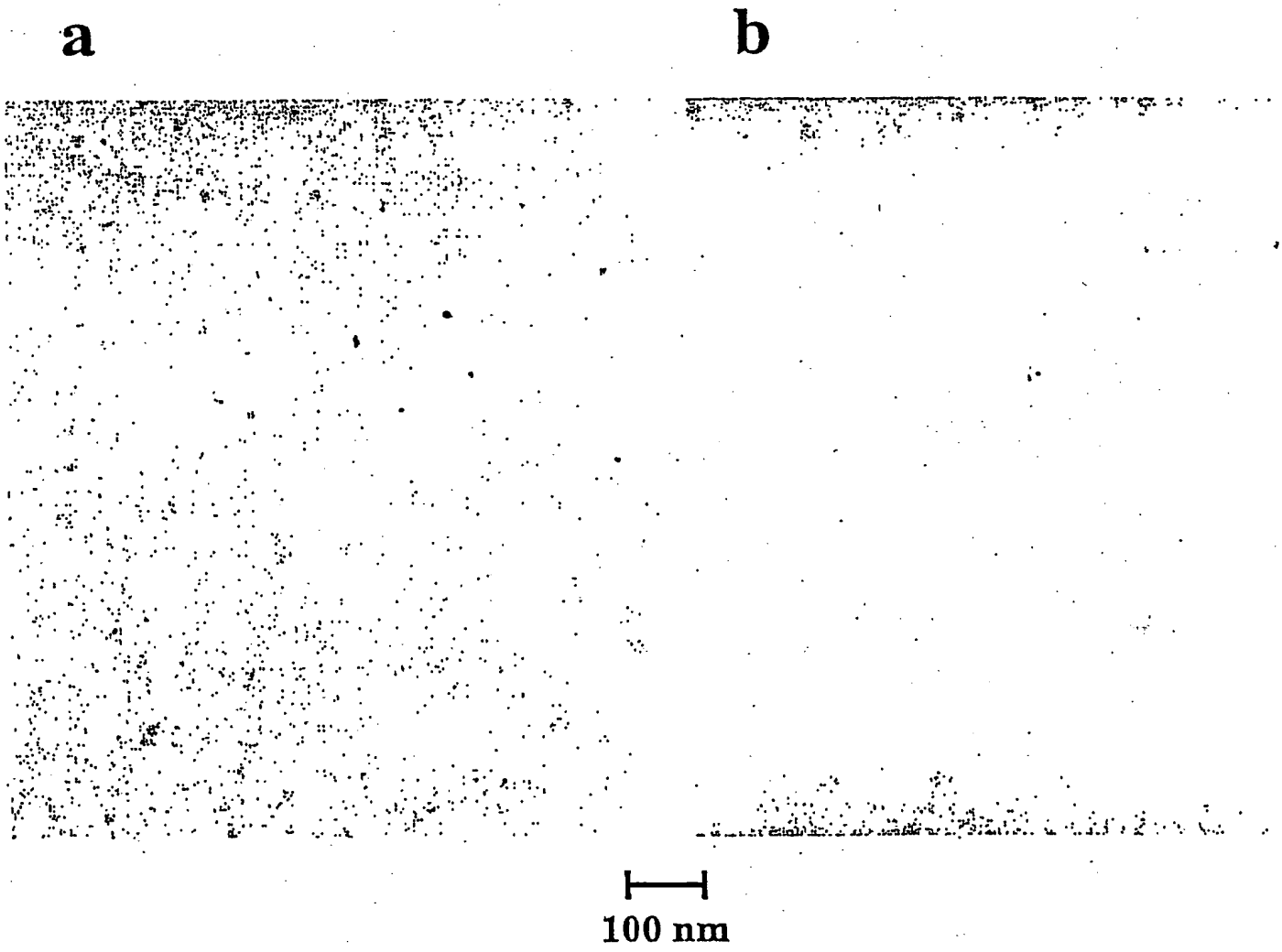


FIGURE 6

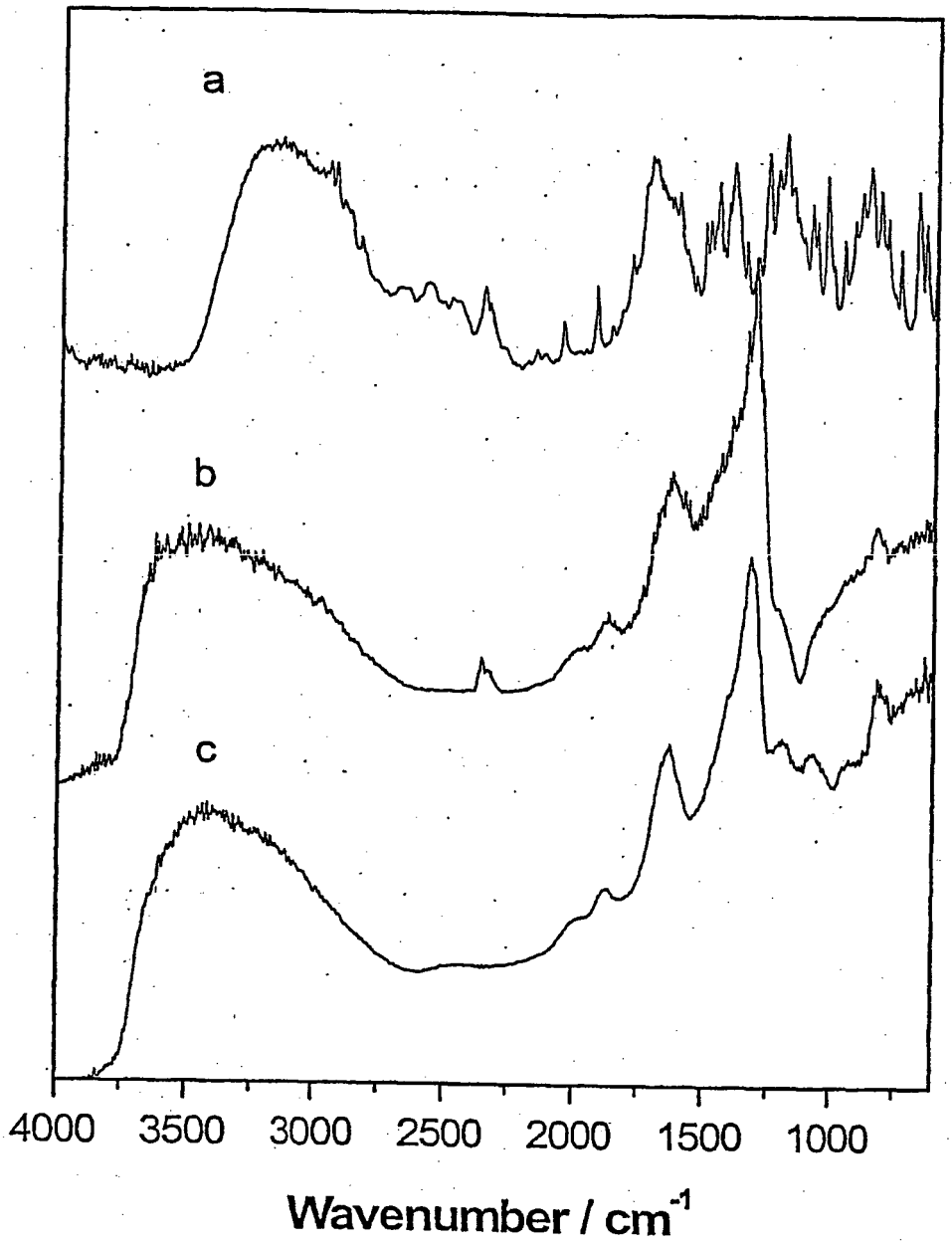


Figure : FTIR spectra of (a) naproxen; (b) Ludox CL (2wt%) + naproxen (0.01 mol dm^{-3}) at pH=8.55 in water; and (c) Ludox CL silica.

FIGURE 7

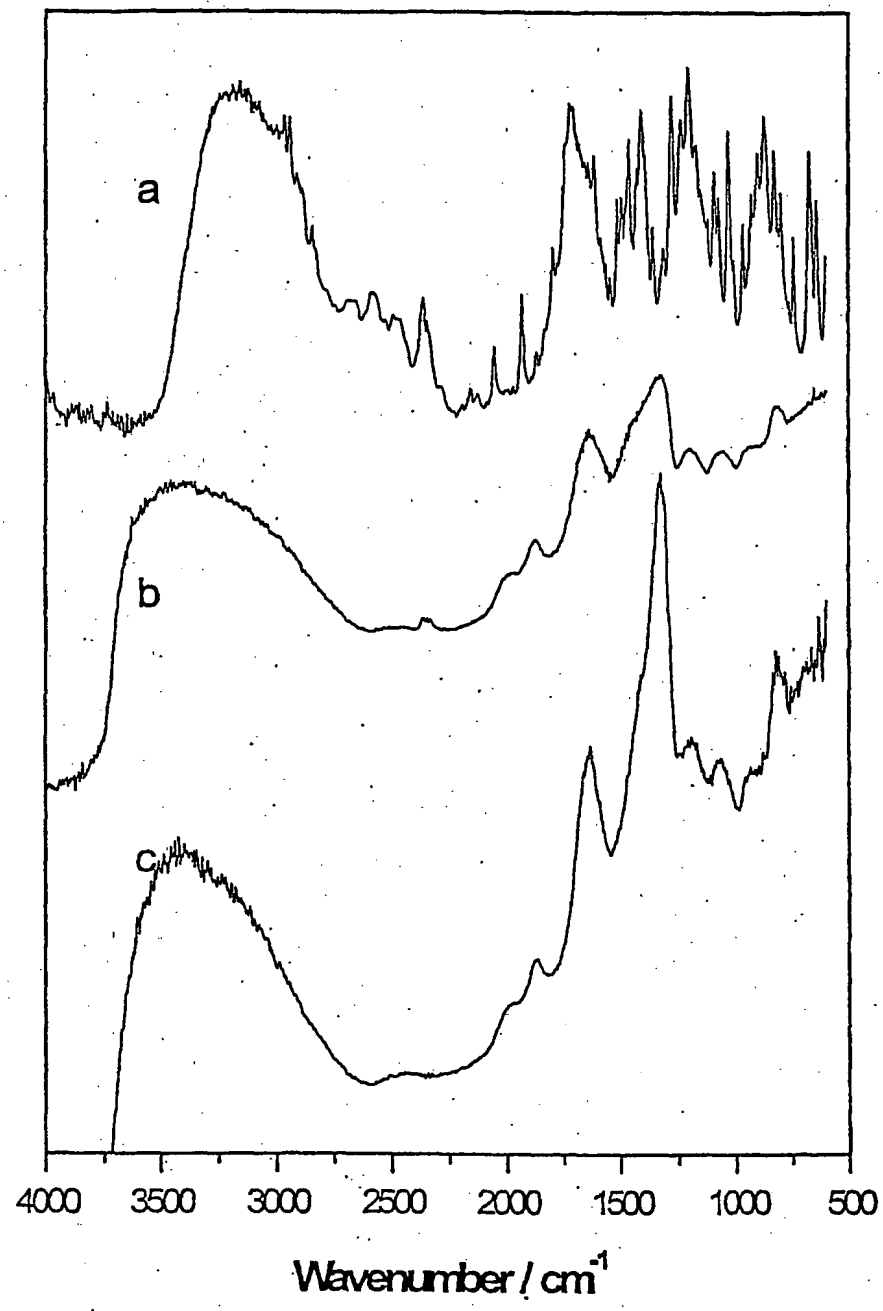


Figure . : FTIR spectra of (a) naproxen; (b) Ludox CL (2wt%) + nap (0.1 mol dm^{-3}) in ethanol and (c) Ludox CL silica.

FIGURE 8

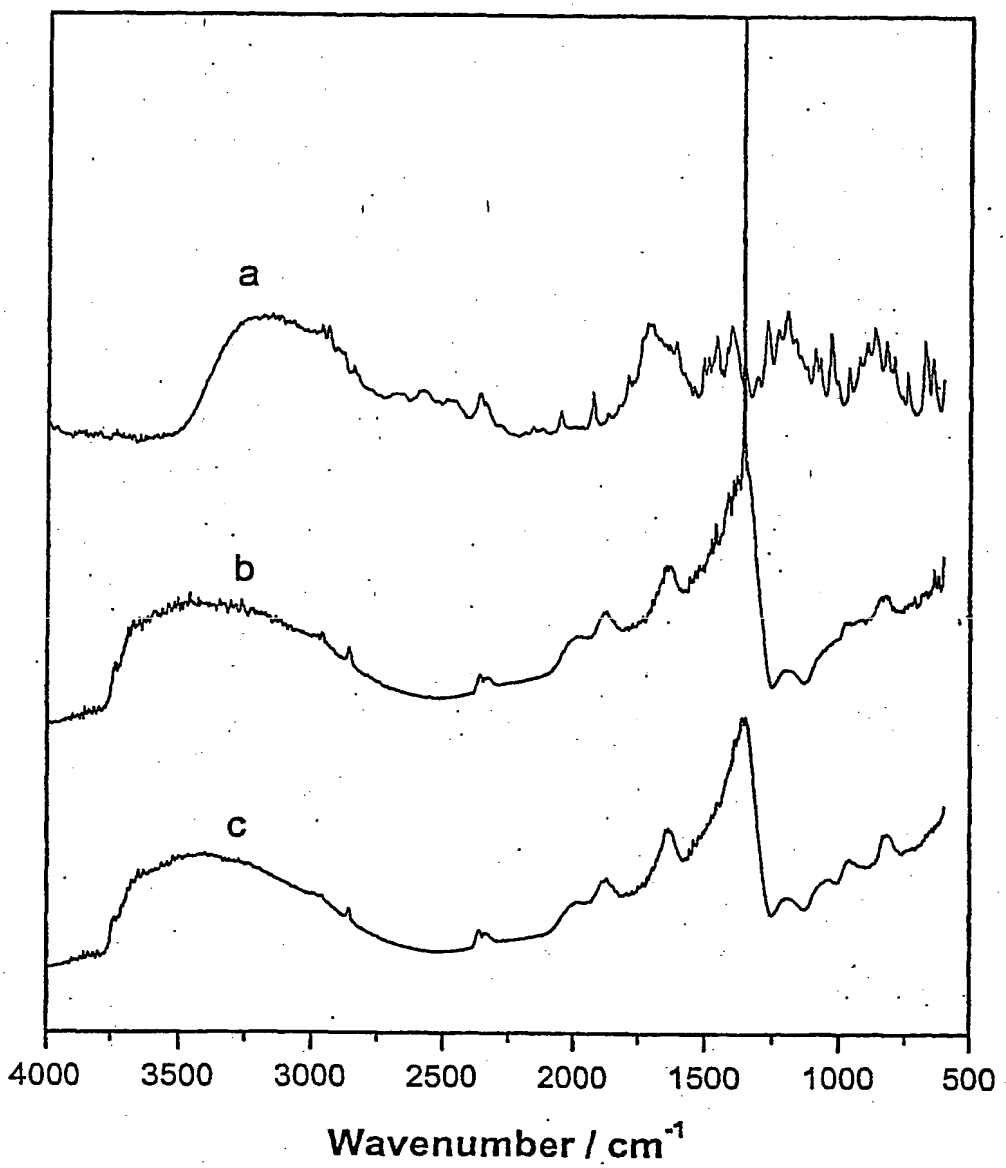


Figure : FTIR spectra of (a) naproxen; (b) Nissan silica (2wt%) + naproxen (0.01 mol dm^{-3}) in methanol and (c) Nissan silica.

FIGURE 9

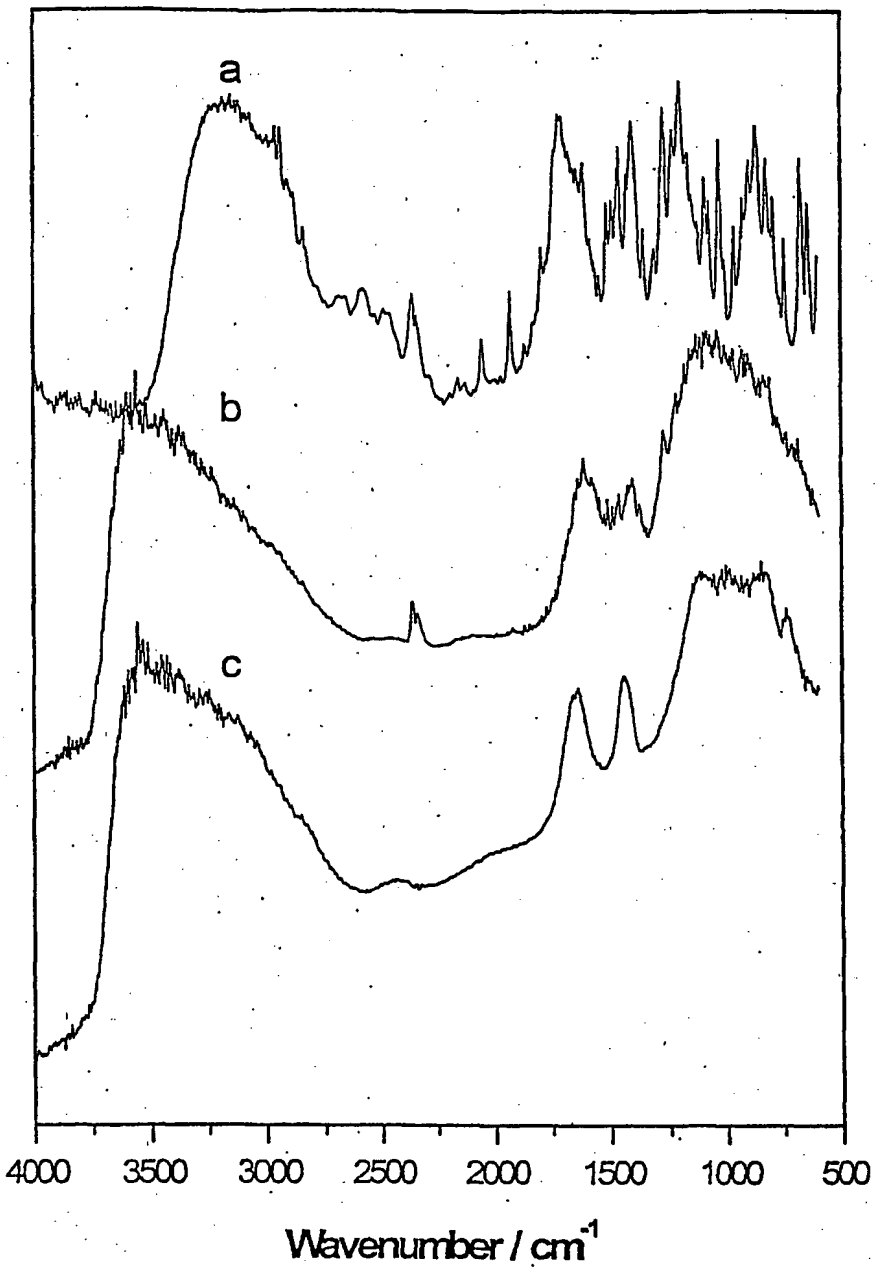


Figure : FTIR spectra of (a) naproxen; (b) Nalco alumina (0.1wt%) + naproxen (0.0025 mol dm^{-3}) in water and (c) alumina Nalco.

FIGURE 10

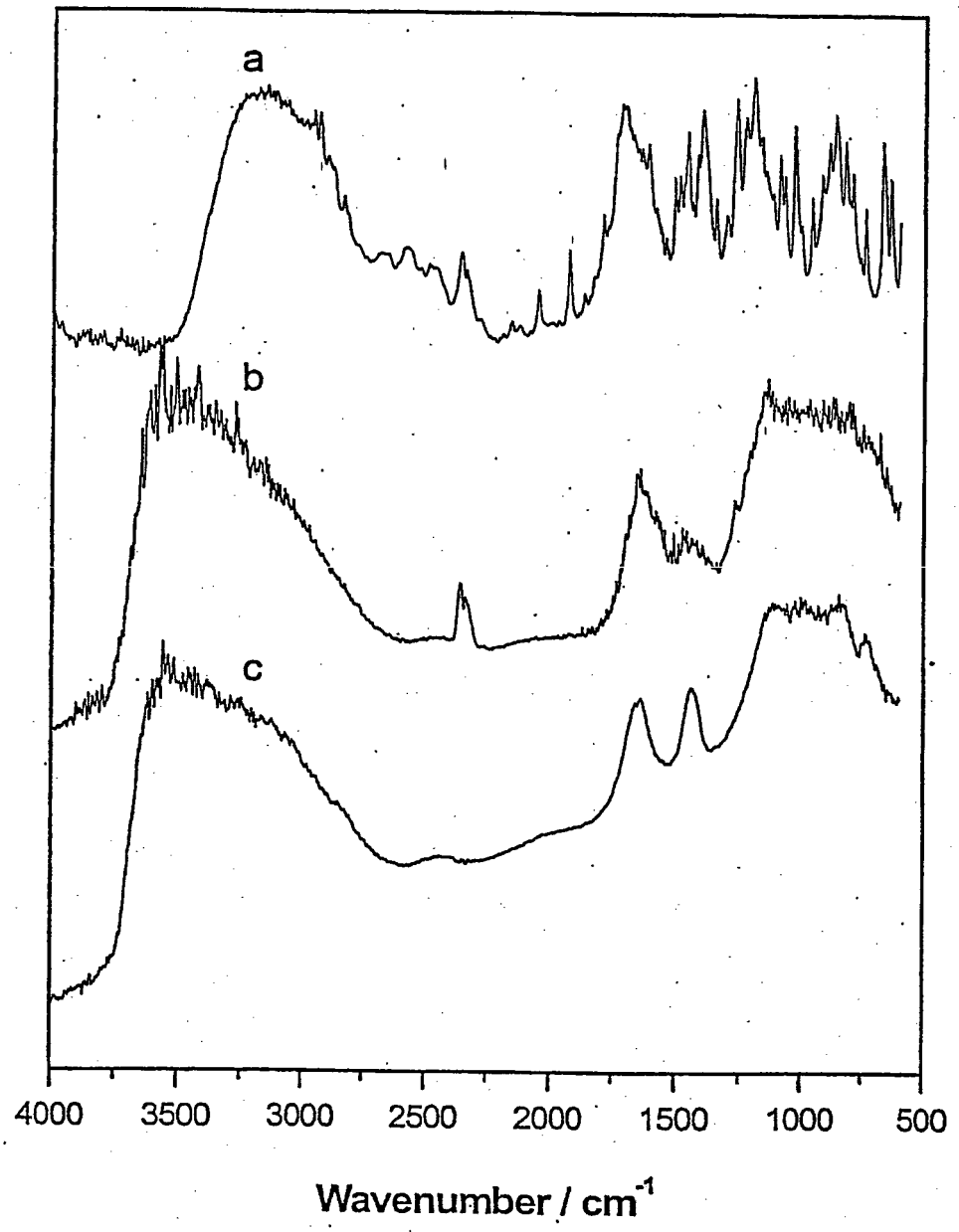


Figure : FTIR spectra of (a) naproxen; (b) Nalco alumina (2wt%) + naproxen (0.05 mol dm^{-3}) in methanol and (c) alumina Nalco.

FIGURE 11

Figure displays the ζ-potentials of both alumina cores as a function of the pH.

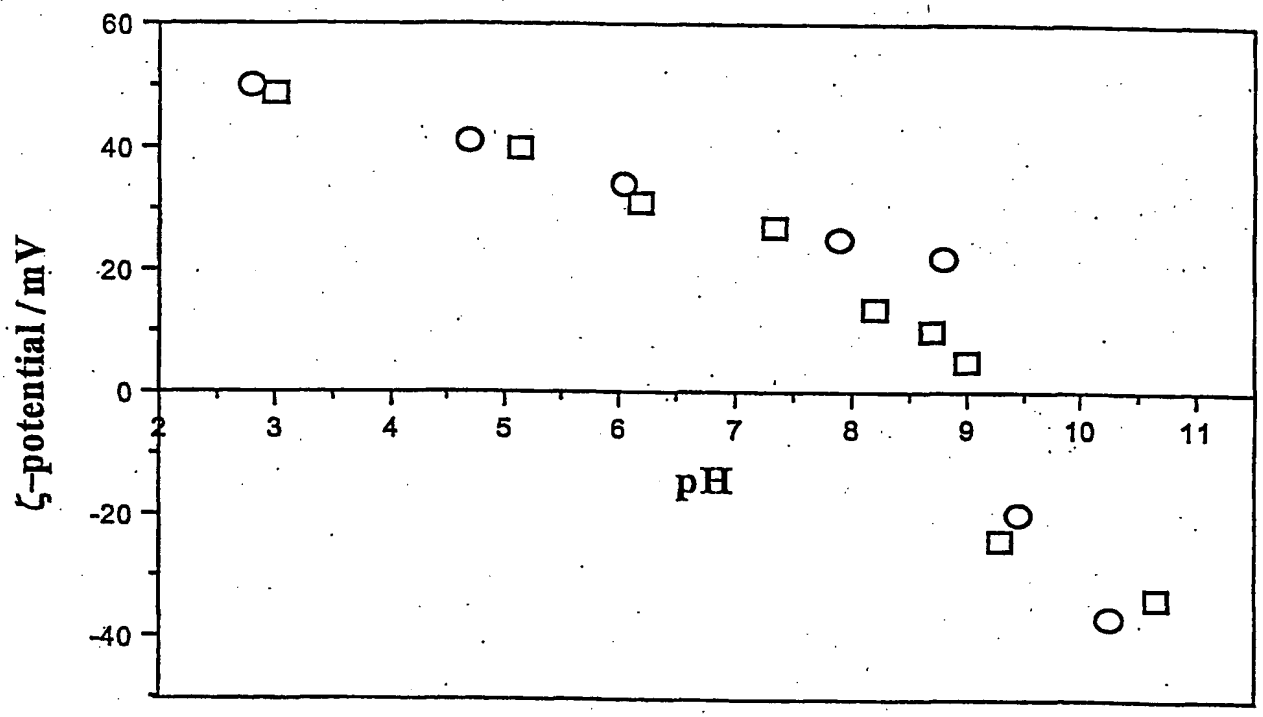


FIGURE 12

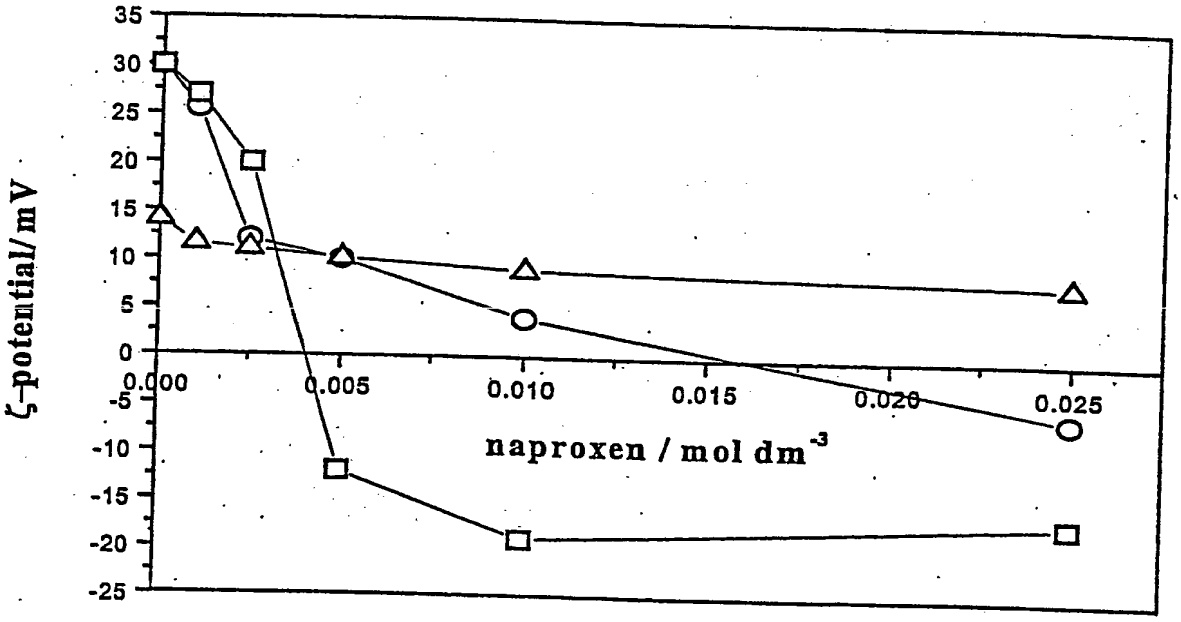


Figure : ζ-potentials of Degussa C (□) Nalco alumina (○) in 1×10^{-3} mol dm^{-3} NaCl aqueous solution at pH = 7.2 ±0.1, and of Degussa C alumina in ethanol (△) as a function of the concentration of added naproxen.

FIGURE 13

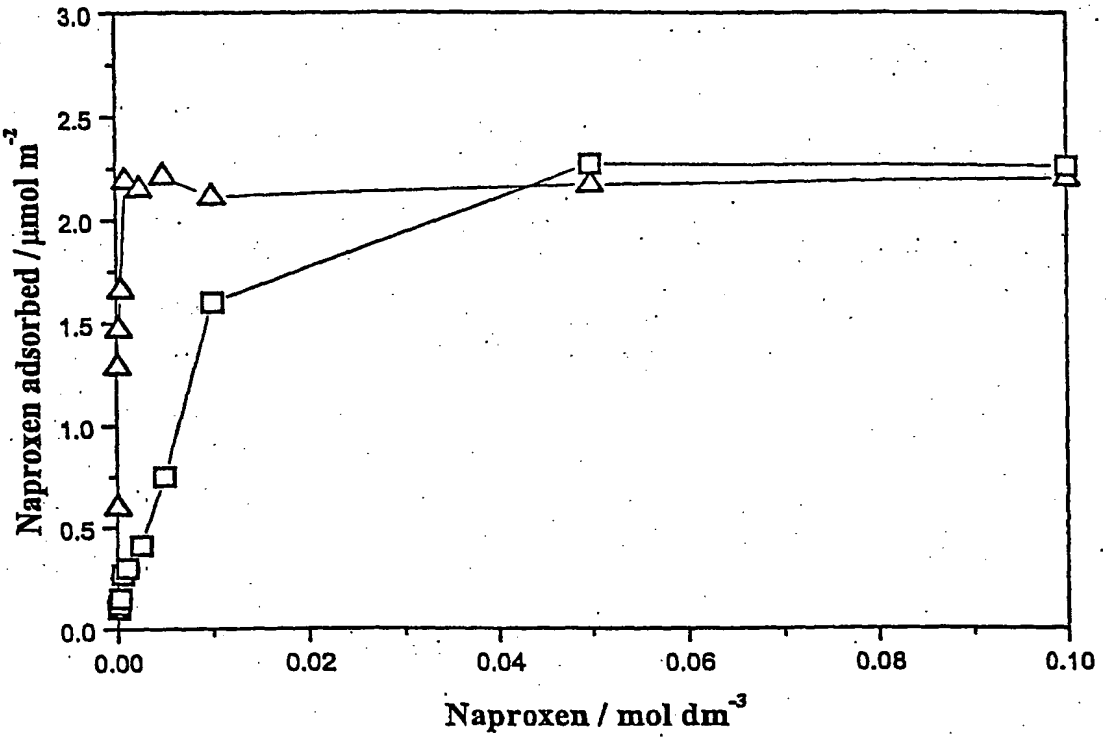


Figure : Adsorption isotherms of naproxen on Degussa C alumina in water (□), pH = 7.2 ±0.1 and in ethanol (▵); Concentration of alumina in all samples was 0.05 wt%.

FIGURE 14

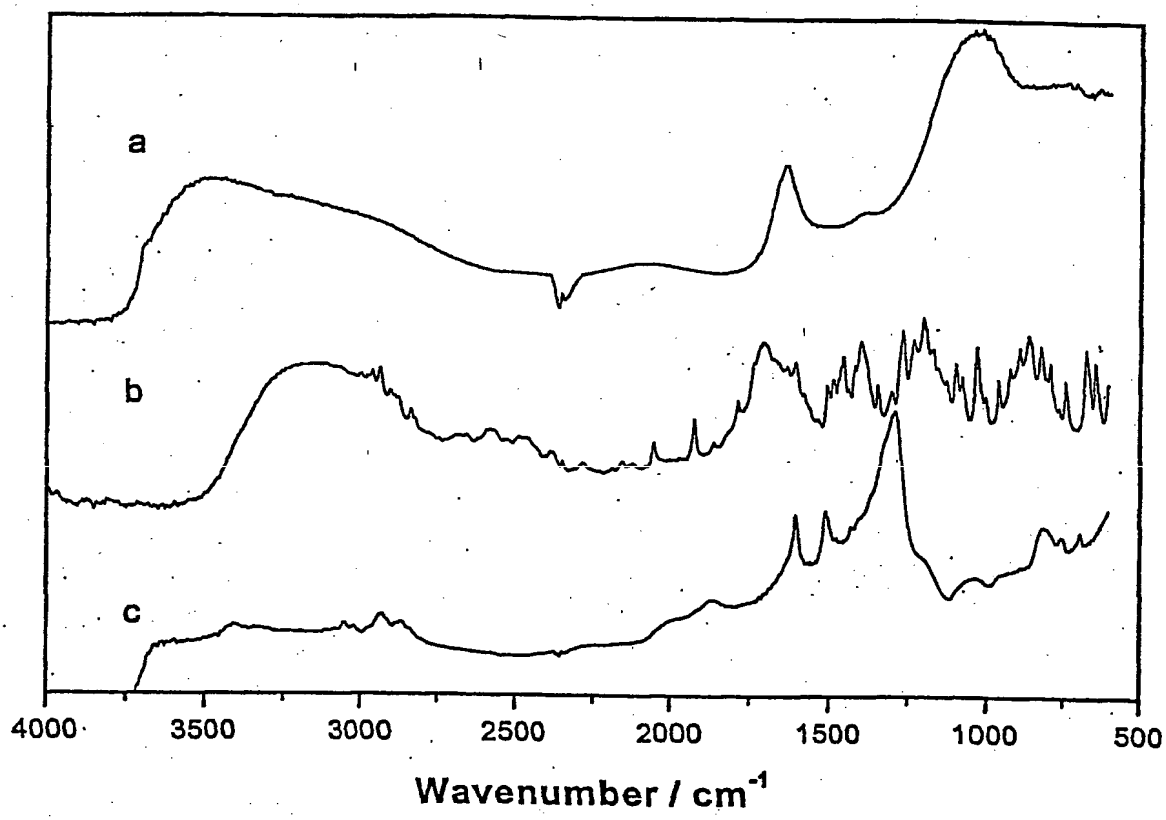


Figure : FTIR spectra of Degussa C alumina (a); Naproxen (b) and Degussa C alumina coated with naproxen in ethanol (c).

FIGURE 15

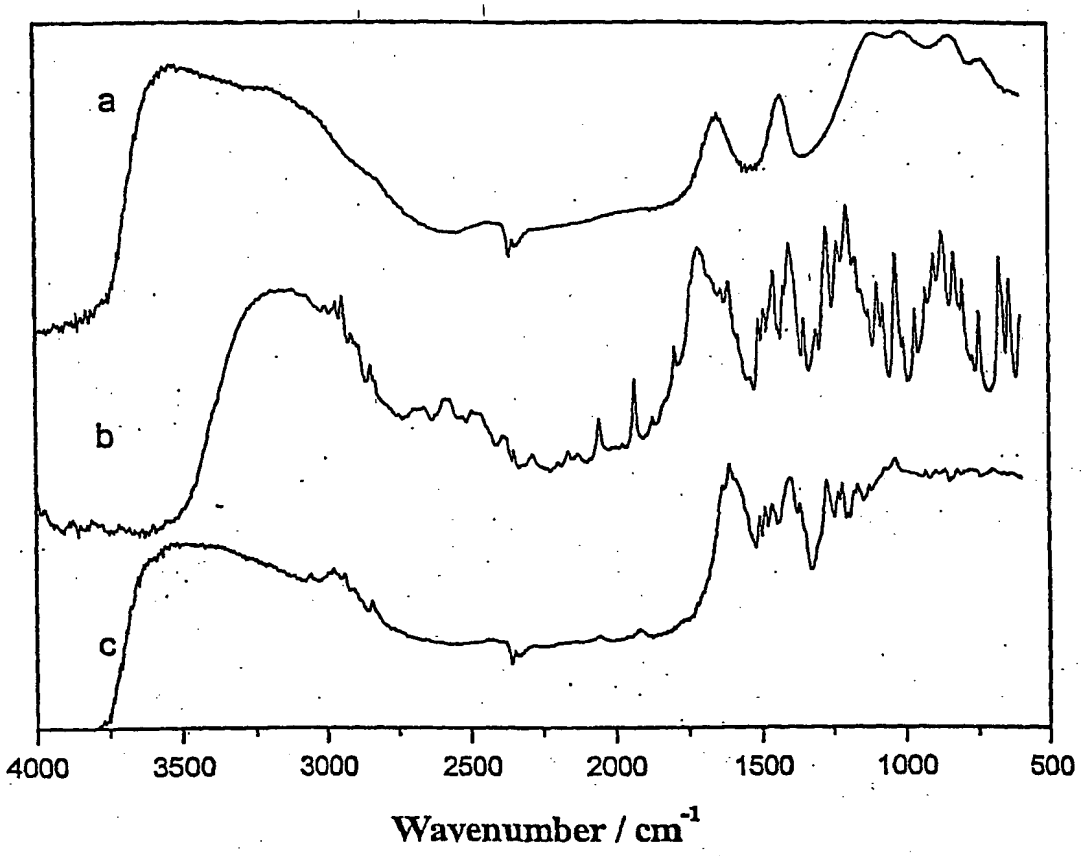


Figure : FTIR spectra of Nalco alumina (a); Naproxen (b) and Nalco alumina coated with naproxen in water (c).

FIGURE 16

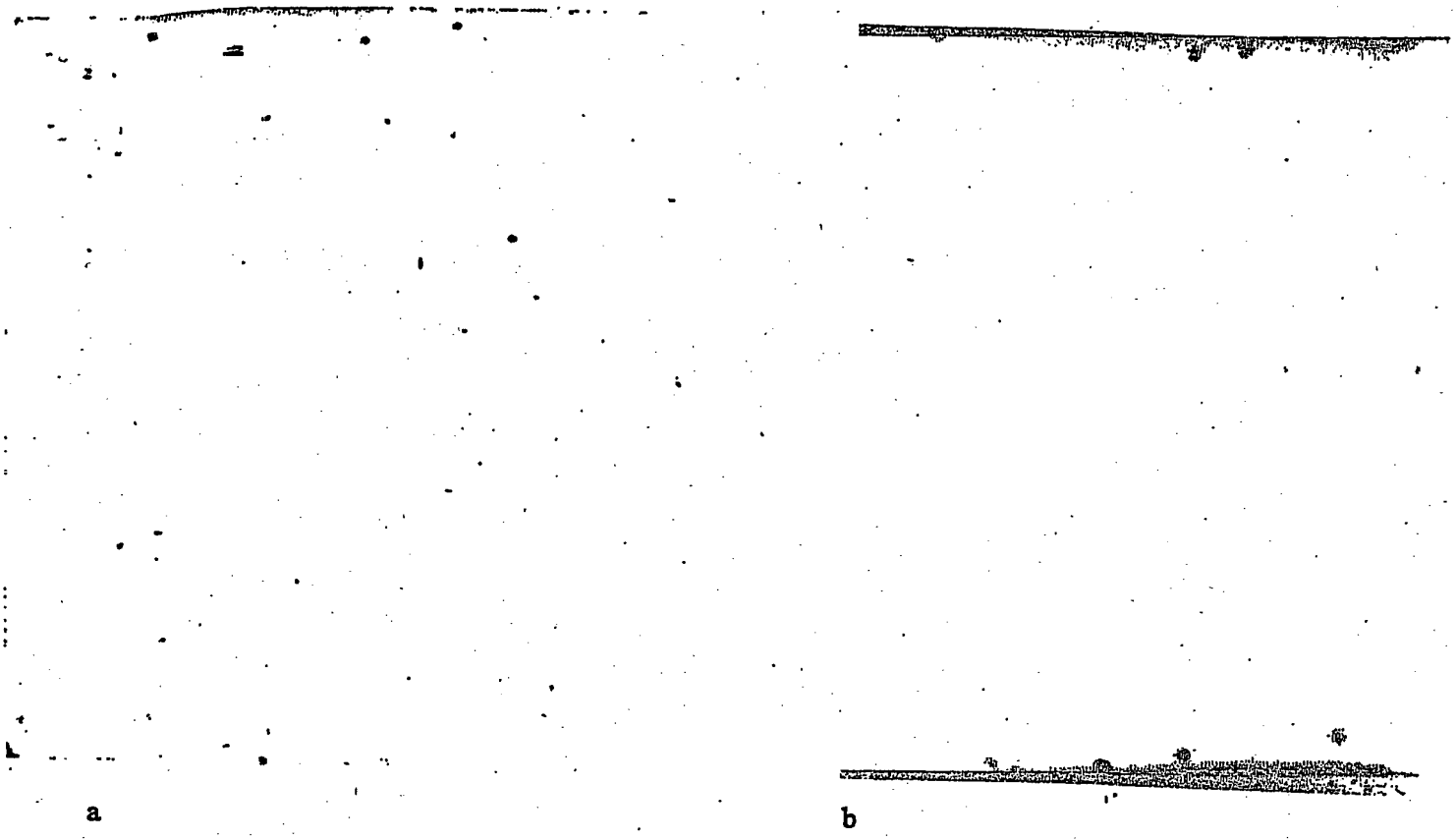
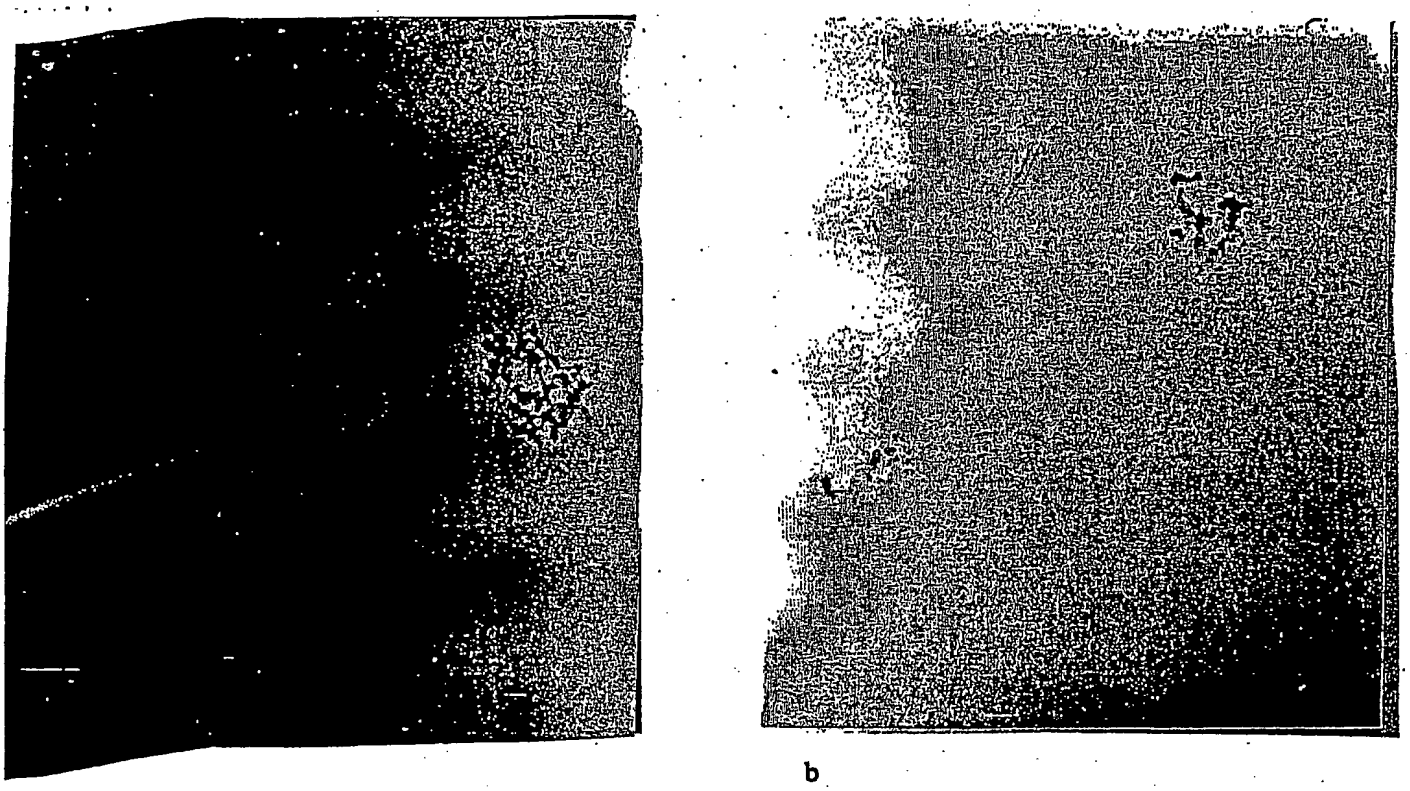


Figure : TEM pictures of Nalco alumina (a) and of Nalco alumina coated with naproxen (b); 1cm≡100 nm

FIGURE 17



b

Figure : TEM pictures of Degussa C alumina (a) and of the same particles coated with naproxen (b); 1cm=100 nm.

FIGURE 18

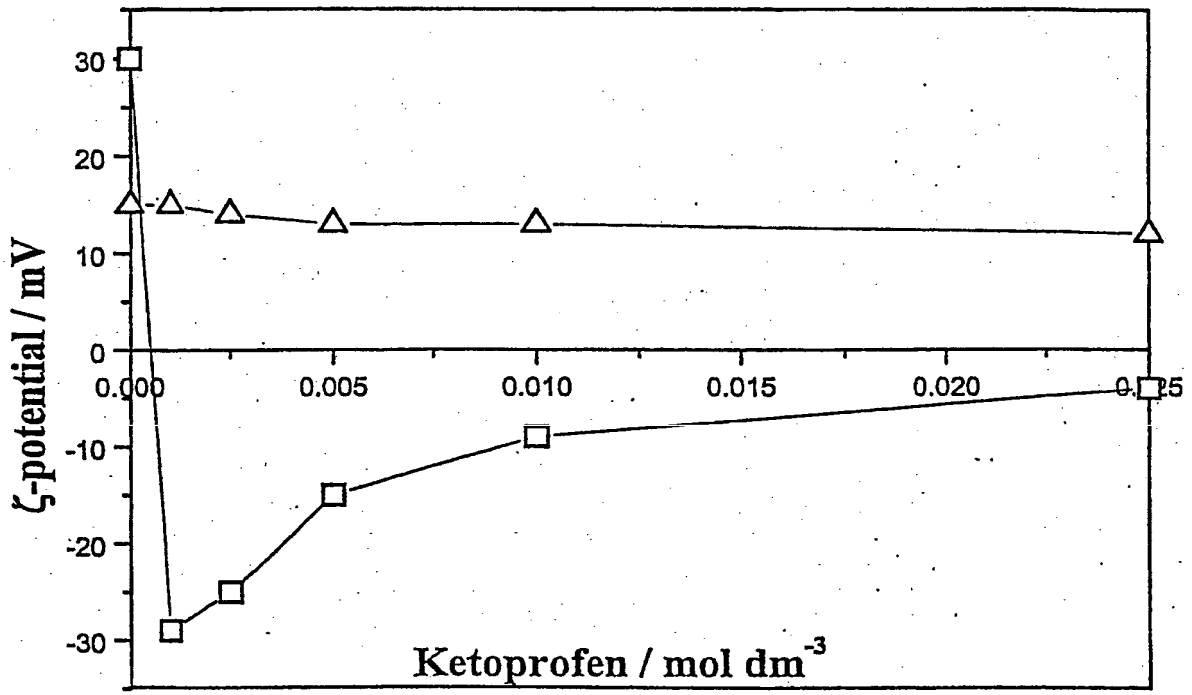


Figure : ζ-potentials of Degussa C alumina (□) in 1×10^{-3} mol dm^{-3} NaCl aqueous solution at pH = 7.2 ±0.1, and of Degussa C alumina in ethanol (△), as a function of the concentration of added ketoprofen.

FIGURE 19

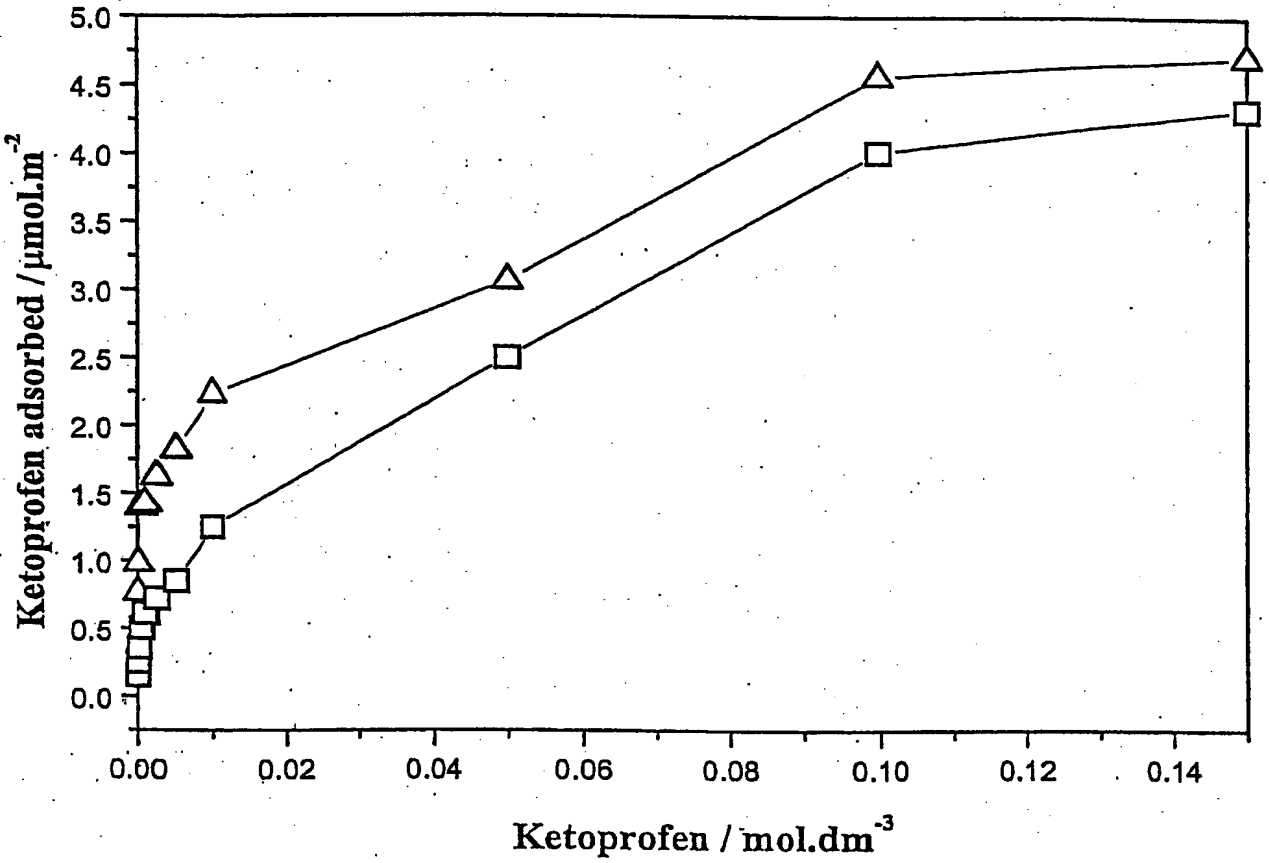


Figure : Adsorption data of ketoprofen on Degussa C alumina in water pH = 7.2 ±0.1 (□), and in ethanol (△); the concentration of alumina in all samples was 0.05 wt%.

FIGURE 20

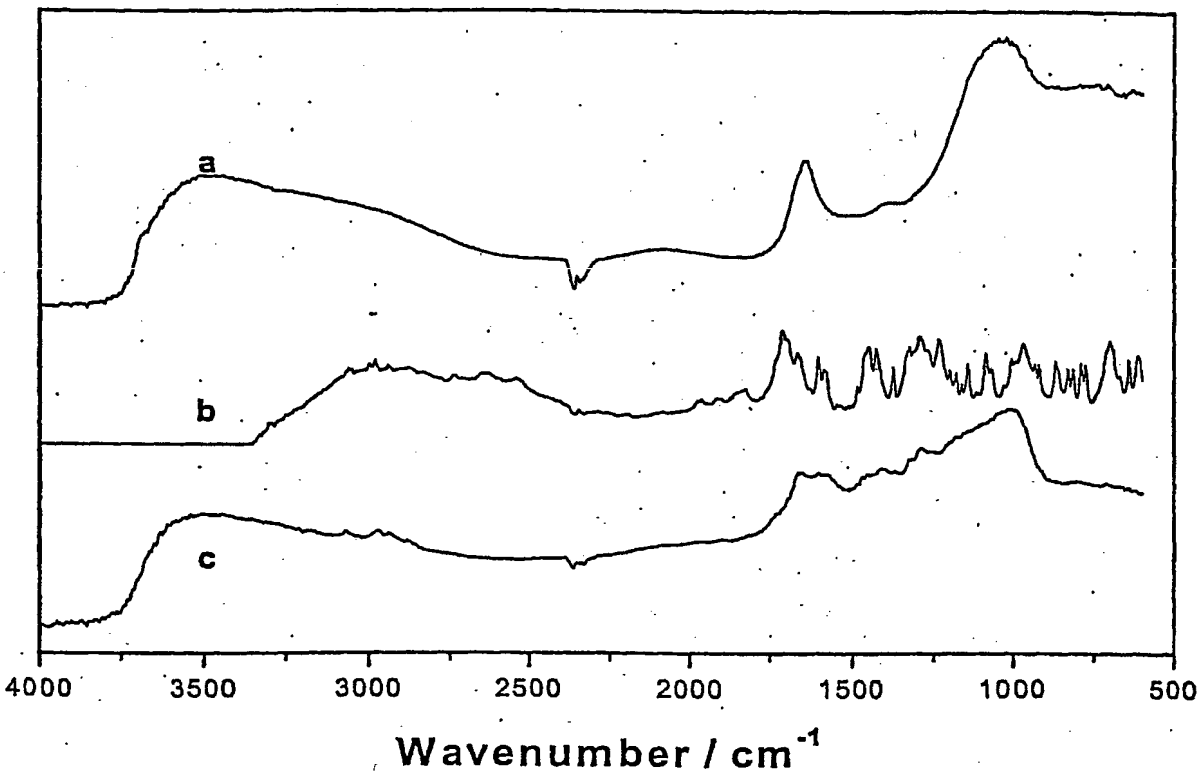


Figure : FTIR spectra of Degussa C alumina (a), ketoprofen (b), and Degussa C alumina coated with ketoprofen in ethanol (c).

FIGURE 21

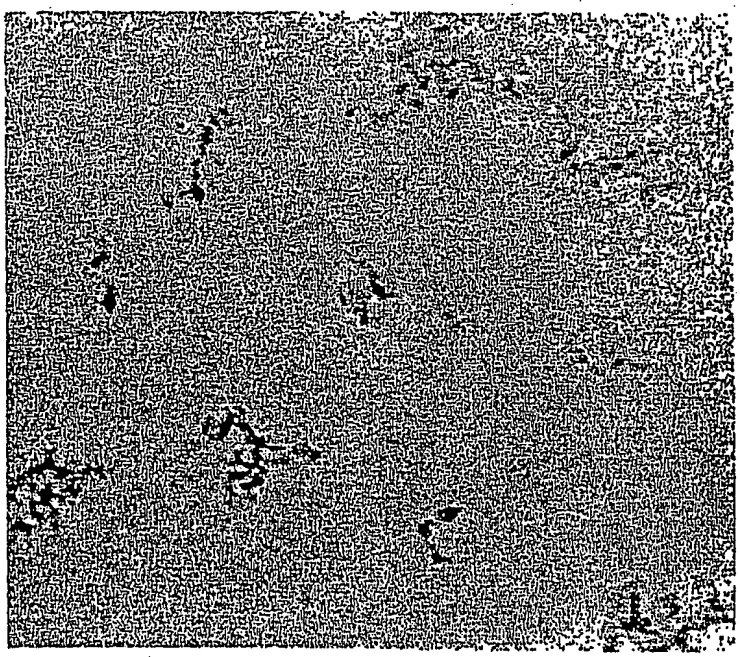


Figure : TEM of Degussa C alumina coated with ketoproen (b); 1cm≡100 nm.

FIGURE 22

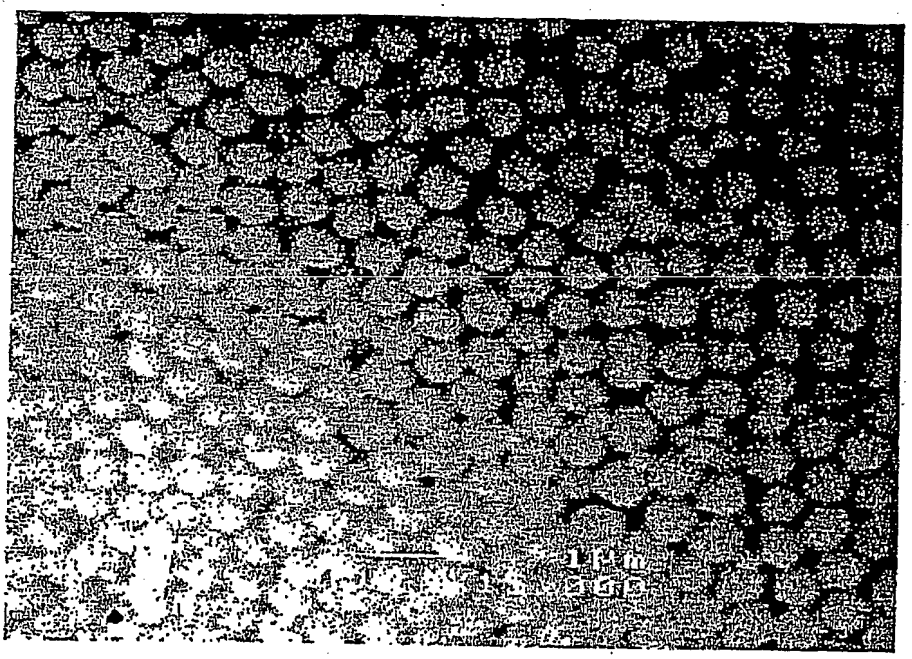


Figure : SEM of silica MP4540

FIGURE 23

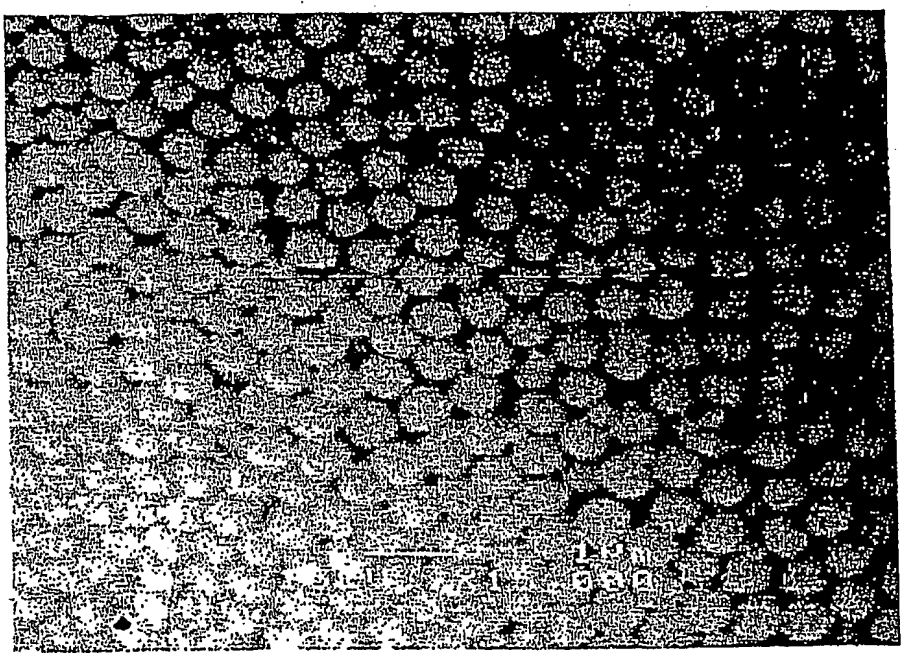
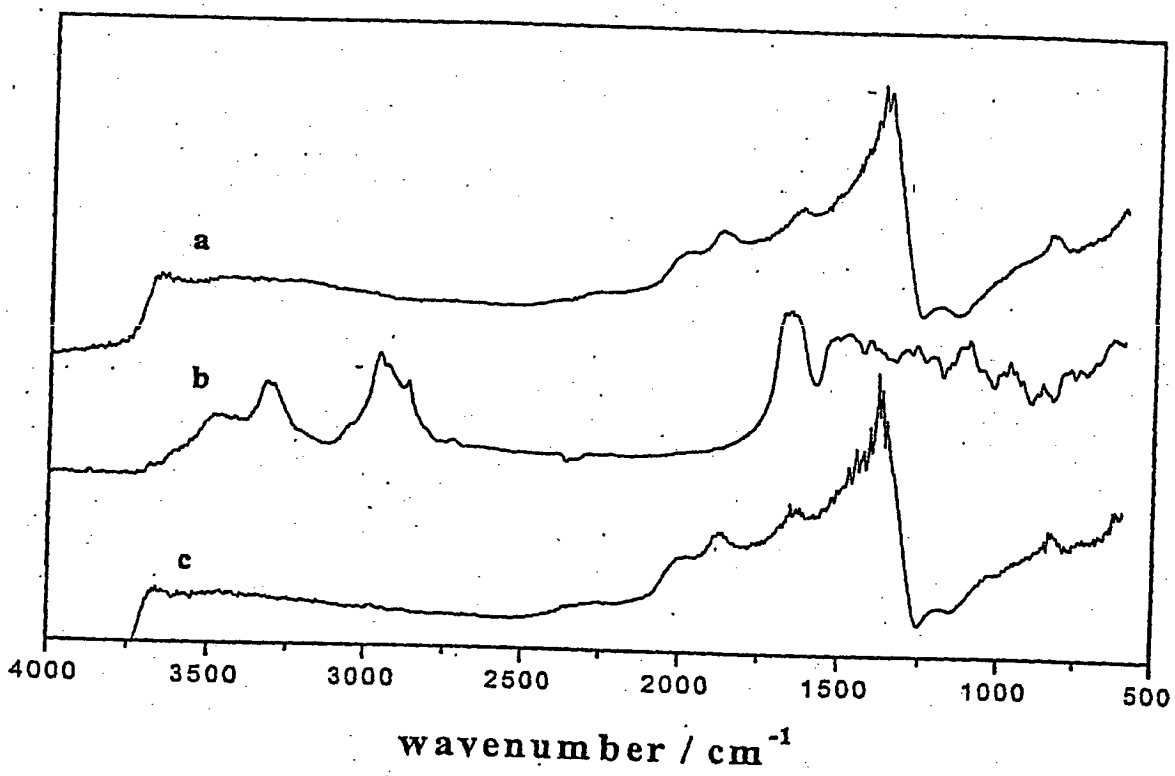


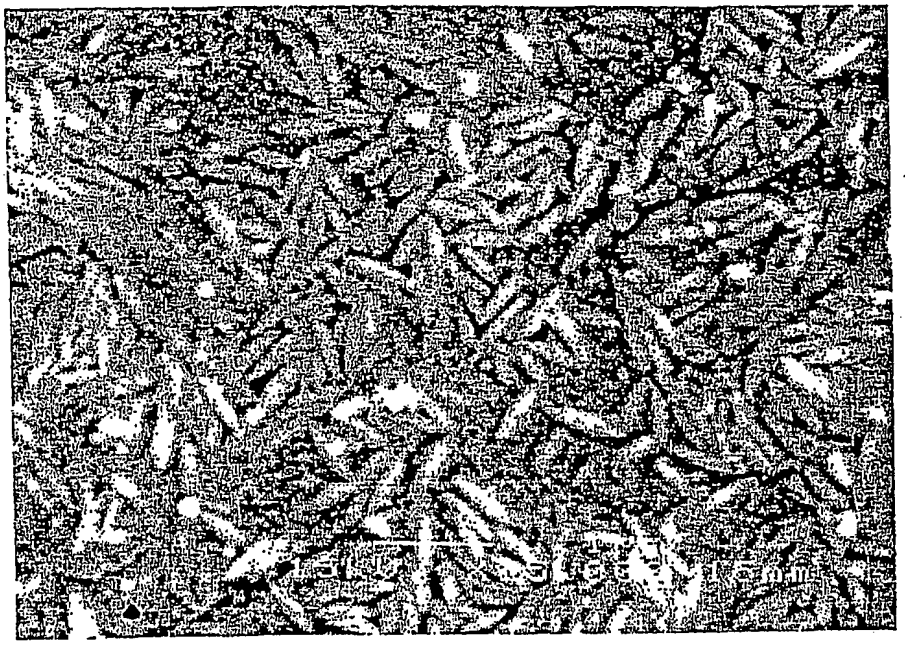
Figure : SEM of Silica (MP4540) equilibrated with cyclosporin (1×10^{-3} mol.dm^{-3}).

FIGURE 24



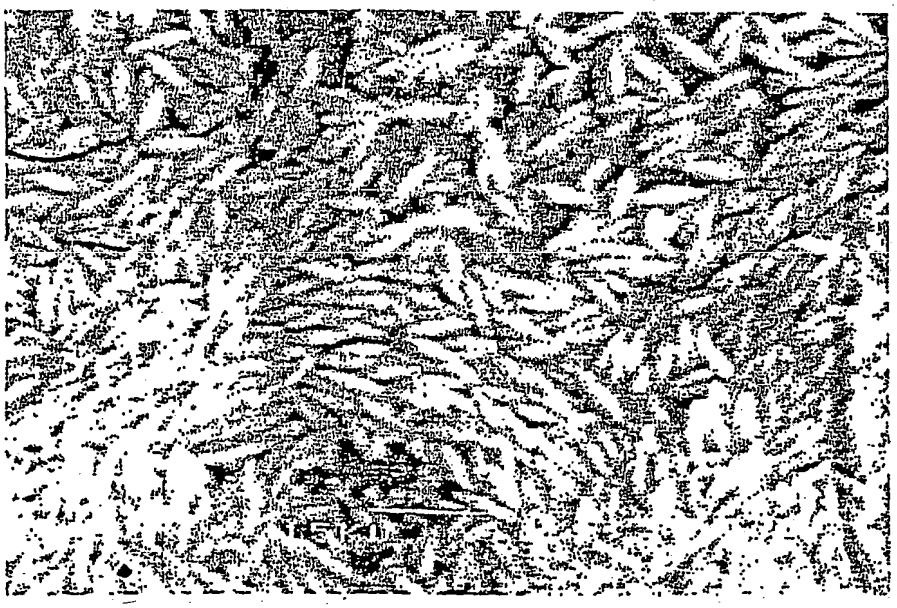
FTIR spectra of silica (MP4540) (a), cyclosporin (b), and silica equilibrated with cyclosporin (1×10^{-3} mol.dm^{-3}) (c).

FIGURE 25



SEM of hematite particles.

FIGURE 26



SEM of hematite after equilibration with cyclosporin ($5*10^{-3}$ $mol.dm^{-3}$).

FIGURE 27

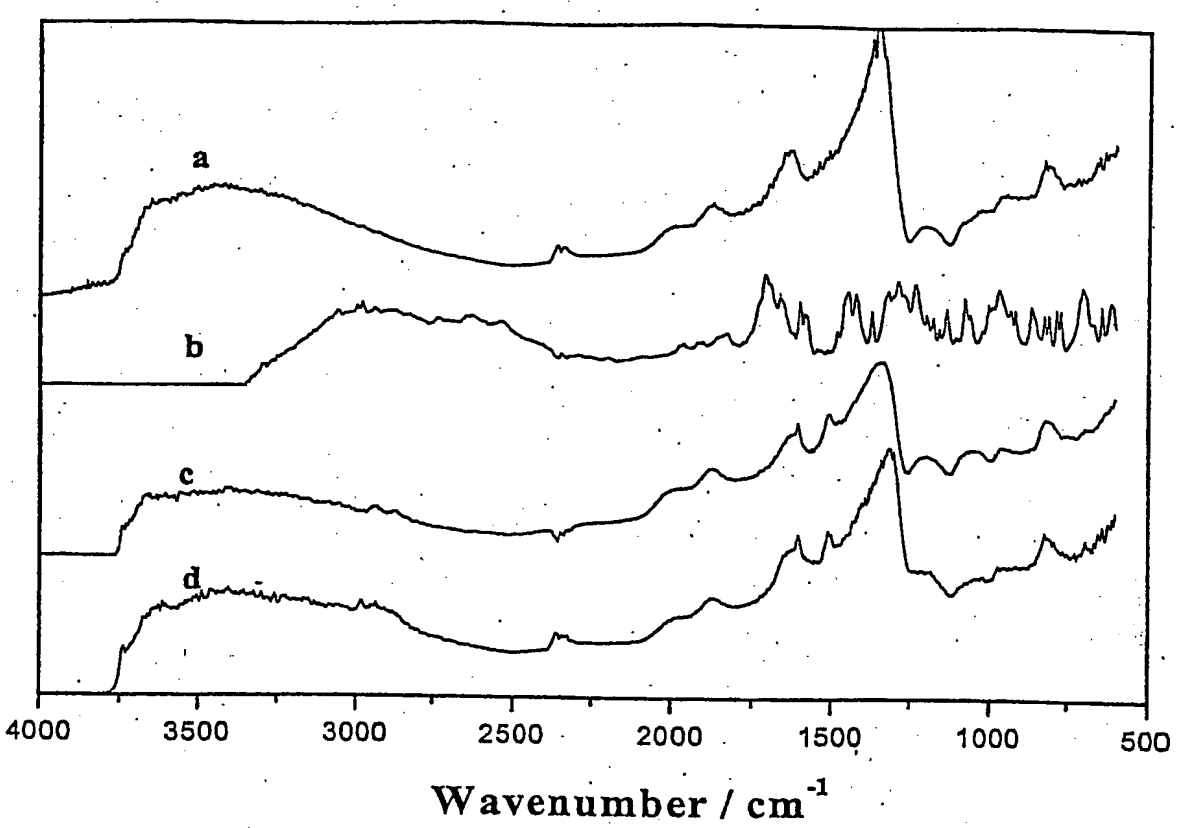


Figure : FTIR spectra of Ludox AM silica (a); ketoprofen (b), modified silica with N-phenylaminopropyltrimethoxysilane (c), and modified silica coated with ketoprofen in water, pH=7.1 (d).

INTERNATIONAL SEARCH REPORT

Interr ıal Application No
PCT/US 02/32619

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K9/51

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, MEDLINE, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	WO 97 13503 A (SELVARAJ ULAGARAJ ;MESSING GARY L (US); PENN STATE RES FOUND (US)) 17 April 1997 (1997-04-17) page 2, line 10 - line 21 page 5, line 12 page 6, line 1 - line 4 page 6, line 16 - line 22 page 7, line 11 - line 15 page 9, line 16 - line 28 page 11, line 17 - line 20 --- -/--	1-14,31, 33,34

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 February 2003	28/02/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Villa Riva, A

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	BILLSTEN PETER ET AL: "Adsorption to silica nanoparticles of human carbonic anhydrase II and truncated forms induce a molten-globule-like structure." FEBS LETTERS, vol. 402, no. 1, 1997, pages 67-72, XP002230383 ISSN: 0014-5793 abstract page 67, right-hand column, line 38 - line 43 page 68, left-hand column, paragraphs 1,3 ---	1-14,31
X	LEIBL H ET AL: "Humoral and cellular immunity induced by antigens adjuvanted with colloidal iron hydroxide" VACCINE, BUTTERWORTH SCIENTIFIC. GUILDFORD, GB, vol. 17, no. 9-10, 1999, pages 1017-1023, XP004158221 ISSN: 0264-410X page 1017, right-hand column, line 15 - line 17 page 1018, right-hand column, paragraphs 1,,2.6 page 1019, right-hand column, paragraph 3.1 page 1021, left-hand column, last paragraph page 1022, left-hand column, line 15 - line 31 ---	1-14,31, 33,34
X	CLARK STEVEN R ET AL: "Fluorimetric investigation of recombinant human growth hormone adsorbed on silica nanoparticles." ANALYTICA CHIMICA ACTA, vol. 290, no. 1-2, 1994, pages 21-26, XP009005241 ISSN: 0003-2670 page 22, line 6 - line 26 ---	1-14,31, 33,34
X	US 5 521 289 A (FURUYA FREDERIC R ET AL) 28 May 1996 (1996-05-28) abstract; examples ---	15-30, 32-34
X	DE 199 12 502 A (INST NEUE MAT GEMEIN GMBH) 21 September 2000 (2000-09-21) page 2, line 36 - line 41 page 2, line 59 -page 3, line 5 examples 1,2; table ---	15-30, 32-34

-/--

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SCHIESTEL T ET AL: "DEVELOPMENT OF A FLEXIBLE CELL TARGETING SYSTEM BASED ON SILICA NANOPARTICLES" MATERIALS RESEARCH SOCIETY SYMPOSIUM PROCEEDINGS, MATERIALS RESEARCH SOCIETY, PITTSBURG, PA, US, vol. 530, 1998, pages 65-71, XP000911914 ISSN: 0272-9172 page 66 figure 1 page 70, last paragraph -page 71, line 10 ---	15-30, 32-34
X	EP 0 556 110 A (EXSYMOL SA) 18 August 1993 (1993-08-18) page 2, line 45 - line 51 page 2, line 1 - line 6 page 4, line 19 - line 24 ---	15-30, 32-34
X	KNEUER C ET AL: "SILICA NANOPARTICLES MODIFIED WITH AMINOSILANES AS CARRIERS FOR PLASMID DNA" INTERNATIONAL JOURNAL OF PHARMACEUTICS, AMSTERDAM, NL, vol. 196, 2000, pages 257-261, XP000900562 ISSN: 0378-5173 the whole document -----	15-30, 32-34

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 02/32619

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 33,34 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte ›nal Application No
PCT/US 02/32619

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9713503	A	17-04-1997	EP	0862420 A1	09-09-1998
			WO	9713503 A1	17-04-1997
US 5521289	A	28-05-1996	US	6121425 A	19-09-2000
			US	6369206 B1	09-04-2002
			US	5728590 A	17-03-1998
DE 19912502	A	21-09-2000	DE	19912502 A1	21-09-2000
			WO	0056288 A1	28-09-2000
			EP	1162955 A1	19-12-2001
EP 0556110	A	18-08-1993	FR	2687071 A1	13-08-1993
			DE	69310229 D1	05-06-1997
			DE	69310229 T2	11-09-1997
			EP	0556110 A1	18-08-1993
			ES	2103441 T3	16-09-1997
			GR	3024283 T3	31-10-1997
			JP	6001724 A	11-01-1994